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# MEASUREMENTS OF *TRYPANOSOMA DIEMYCTYLI* FROM DIFFERENT HOSTS AND THEIR RELATION TO SPECIFIC IDENTIFICATION, HEREDITY AND ENVIRONMENT

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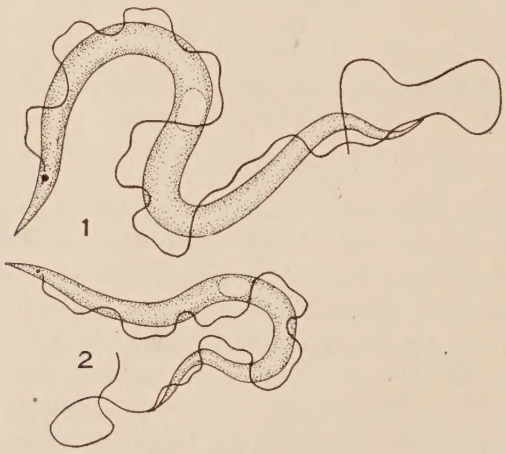
## INTRODUCTION

The problems involved in studies of strains of trypanosomes are both scientific and practical. Considerable time and effort have been devoted to attempts to find size differences between human trypanosomes of supposedly different species. Investigators have endeavored also by means of measurements to establish the identity of human trypanosomes with those of certain lower animals and thus to locate animal reservoirs. Furthermore, the data resulting from these researches throw some light upon the possibility of the existence of heritably diverse strains within a species, and upon the effects of the environment, *i. e.* the blood stream, of different species of hosts and of different hosts of the same species on a single strain. It is this last problem that is considered in the following pages.

*Trypanosoma diemyctyli* (Figs. 1 and 2) was found by Tobey in 1906 to be present in all of a number of newts purchased in an animal store in Boston. During the spring months of 1919 the writer examined the blood of six different species of salamanders. In none of these were any organisms found except in *Diemyctylus viridescens*. All of seventy-eight aquatic specimens of these were infected and two out of seven land specimens. The negative animals were 46 specimens of *Necturus maculosus*, six of *Plethodon glutinosus*, five of *P. cinereus*, 11 of *Desmognathus fusca*, and six of *Spelerpes bilineatus*.

The difference between the aquatic and land forms of *Diemyctylus viridescens* as regards infection with trypanosomes presents an interesting problem. The life cycle of these amphibia includes a year in the water, then a second year on land, and finally a return to the water for mating in the third year. Evidently while on land the infection with the trypanosomes is much decreased. The small numbers of

organisms found in these terrestrial specimens may be due to the absence on land of the transmitting agent, which is unknown. The universal abundance of the trypanosomes in the aquatic specimens may be due to their continued inoculation with young stages that have developed in the intermediate host; and the lesser numbers in the land forms, to a gradual dying out of older trypanosomes. It is of interest in this connection to note that of 34 "land" frogs examined during the spring and summer of 1919 only two were infected with trypanosomes whereas of 41 "water" frogs 28 were infected.




EXPLANATION OF FIGURES

Fig. 1.—Typical specimen of *Trypanosoma diemyctyli* from newt 19. × 1600.
Fig. 2.—Typical specimen of *Trypanosoma diemyctyli* from newt 15. × 1600.

## METHODS OF MEASURING TRYPANOSOMES

In any investigation involving measurements the accuracy of the results depends primarily on the accuracy of the measurements. Trypanosomes are difficult to measure precisely, since their bodies are almost always thrown into curves when fixed. This is especially true of long slender forms. Several methods of obtaining accurate measurements have been employed by investigators.

The method adopted by Bruce, Hamerton and Bateman in 1909 was to draw an outline of each specimen with a camera lucida at a magnification of 2000 diameters "and then to measure along the middle line of the body by means of a pair of fine compasses, the points of which are separated 2 mm. Each step the compass takes is therefore equal to 1 micron."

A modification of this method was employed by Stephens and Fantham (1912). They projected the trypanosomes on a screen with

a microprojection apparatus and then traced their outlines with a sharp pencil. A magnification of 2500 diameters was adopted. The drawings were then measured by placing over them semitransparent tracing paper on which a straight line was drawn in ink. One end of the ink line was placed on one end of the drawing and rotated whenever the axis of the trypanosome curved. When the end of the drawing was reached the distance was measured with a millimeter scale.

The method used by the writer seems more desirable than those described above. The trypanosomes were projected with a camera lucida upon a drawing card at a magnification of 1600 diameters. The anterior and posterior ends and kinetonucleus were then indicated with a dot; the width of the body at the nucleus was recorded by two short parallel lines; and the nucleus was drawn. A single line was then drawn down the center of the body from the posterior end to the anterior end. With a chartometer or "map measurer" the distances were easily and accurately obtained.

## INVESTIGATIONS INVOLVING TRYPANOSOME MEASUREMENTS

Bruce and his colleagues have measured thousands of trypanosomes of various species in their endeavor to distinguish by size characteristics between the species pathogenic in man and those that occur in the lower animals. The organisms measured were derived from various strains and were taken from a number of species of both wild and laboratory animals. Bruce finally decided that this method of specific identification could not be depended on.

Data regarding the effects of different hosts on the size of the specimens have been provided by various investigators. Thus Laveran and Mesnil (1912) noted a difference between the length of specimens of *T. brucei* grown in the horse and those grown in rodents. Duke (1912) has suggested that strains of numerous varieties exist among trypanosomes of any species, and that alterations in the morphology of a strain may follow continued passage through laboratory animals.

It seems probable from the work of Miss Robertson (1912) on *T. gambiense* that one difficulty in biometric studies of such trypanosomes, is the presence of an endogenous cycle in mammals which results in changes in the types at intervals that cannot be determined by the date of infection. Even if diversities in size were noted in specimens from different hosts the results would not be conclusive. It seems, therefore, that measurements of these polymorphic species are of doubtful value and that better results may be expected when monomorphic forms are studied.

Pearson (1914) has made a biometrical study of many of the measurements published by other investigators and concludes that actual statistical analysis does not in any way confirm the bulk of the

conclusions reached by Sir David Bruce and his collaborators. He points out the fact that the data available do not provide material for an analysis of the relative influence of the various environmental factors and hence one cannot determine whether divergences indicate different strains or merely modifications due to different environments.

Most of the quantitative studies of trypanosomes deal with attempts to secure data that will provide means of specific diagnosis. Those data that might furnish evidence of diversities due to the character of the host in which the specimens were grown are of doubtful value because most of the species studied have been dimorphic or polymorphic and hence have exhibited great variations even from a single host. Furthermore, many of the strains measured had received dissimilar treatment; some were taken directly from wild animals, whereas others had been passed through series of laboratory animals of different species during periods of varying length. This treatment may have had an influence on the morphological characteristics of the strains used. The available measurements of monomorphic trypanosomes do not exhibit variations that make possible any definite conclusion as regards diversities when grown in different hosts.

A review of the literature, especially the paper by Pearson, emphasizes the importance of more careful studies of the relations between trypanosomes and their environment represented by different species of hosts and by different individuals of one host species. It is possible to isolate single trypanosomes and to obtain in one host animal a supply of specimens that can be compared with the descendants of other single specimens in host animals of the same or other species. Work of this character is now in progress in this laboratory.

## TRYPANOSOMA DIEMYCTYLI FROM DIFFERENT HOSTS

Measurements were made of 100 specimens of *T. diemyctyli* that were taken at random, ten from each of ten individuals of *Diemyctylus viridescens*. Some of these measurements are presented in Tables 1 and 2. The preparations were all made on the same day and stained with Wright's stain. No selection was made either of the newts from which the trypanosomes were obtained or of the trypanosomes on the slides. The first ten trypanosomes that were found in the preparation from each newt were drawn at a magnification of 1,600 diameters and then measured with a map measurer.

Table 1 includes the variations in the distances from the anterior end of the body to the center of the nucleus, from the center of the nucleus to the kinetonucleus,* and from the kinetonucleus to the posterior end. The width of the body at the point where the nucleus is

* By the kinetonucleus is meant the body called by the French centrosome, by the Germans blepharoplast, and by certain Americans parabasal.

situated is also given as well as average distances. The data are arranged in a series with those of the largest set of ten trypanosomes at the top of the table and those of the smallest set of ten at the bottom. The final averages differ somewhat from those given by Tobey. Tobey's specimens were from 45 to 50$\mu$ in length with a flagellum 24$\mu$ long. My specimens ranged from 42.5 to 75.3$\mu$ in length with a flagellum 32$\mu$ long. The measurements of the trypanosomes from newts 19 and 15 are particularly interesting. The data show that in *every one* of the trypanosomes from newt 19 the distance from the anterior end to the center of the nucleus is considerably greater than it is in *any* of the specimens from newt 15. This distance in newt 19 ranges from 31 to 44$\mu$, whereas in newt 15 it ranges from 20 to 23$\mu$. The average in the ten specimens from newt 19 is 36.3$\mu$ and in newt 15, 21.4$\mu$, a difference of over 50 per cent. The differences between the distances from the center of the nucleus to the kinetonucleus are similar but not so great, those of the trypanosomes from newt 19 being only 25 per cent. greater than from newt 15. The distances from kinetonucleus to posterior end show differences averaging over 50 per cent., and the average difference in total length exclusive of the flagellum is approximately 50 per cent.

TABLE 1.—VARIATION IN LENGTH AND WIDTH OF 100 SPECIMENS OF *T. diemyctyli* TAKEN AT RANDOM, TEN FROM EACH OF TEN NEWTS, AND THE AVERAGE LENGTH AND WIDTH OF THE SAME GROUPS OF TEN. ALL MEASUREMENTS IN MICRONS

| Number of Newt | Anterior End of Body to Center of Nucleus | | Center of Nucleus to Kinetonucleus | | Kinetonucleus to Posterior End | | Total Length Exclusive of Flagellum | | Width of Body at Nucleus | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Variation | Average | Variation | Average | Variation | Average | Variation | Average | Variation | Average |
| 19 | 31–44 | 36.3 | 23–32 | 25.6 | 5.6–6.9 | 6.1 | 61.6–75.3 | 68.0 | 3.1–3.8 | 3.3 |
| 20 | 32–42 | 35.5 | 23–29 | 26.1 | 5.0–6.9 | 5.9 | 58.6–72.9 | 66.5 | 2.5–4.4 | 3.5 |
| 14 | 28–33 | 30.5 | 26–29 | 27.7 | 3.1–5.6 | 4.3 | 59.4–66.6 | 62.5 | 2.5–3.1 | 2.9 |
| 18 | 25–36 | 27.9 | 22–27 | 23.9 | 3.8–5.0 | 4.3 | 50.8–65.4 | 56.1 | 2.5–3.8 | 2.9 |
| 16 | 22–31 | 27.2 | 20–30 | 23.9 | 3.1–5.6 | 3.9 | 45.1–65.4 | 55.0 | 2.5–3.1 | 2.6 |
| 10 | 23–31 | 26.4 | 21–26 | 23.9 | 3.8–4.4 | 4.3 | 48.8–60.0 | 54.6 | 3.1–4.4 | 3.4 |
| 17 | 21–27 | 24.1 | 22–25 | 24.0 | 3.1–3.8 | 3.5 | 48.1–55.8 | 51.6 | 2.5–3.1 | 2.7 |
| 12 | 23–29 | 25.1 | 21–25 | 22.9 | 2.5–3.8 | 3.3 | 38.1–57.1 | 51.3 | 2.5–3.8 | 3.0 |
| 9 | 19–29 | 24.4 | 18–27 | 21.8 | 3.1–4.4 | 3.6 | 45.0–60.3 | 49.7 | 1.9–3.1 | 2.6 |
| 15 | 20–23 | 21.4 | 19–22 | 20.6 | 2.2–3.4 | 2.7 | 42.5–48.4 | 44.7 | 1.9–2.5 | 2.2 |

The striking fact brought out by a comparison of these data is the large and constant difference in length between the two sets of trypanosomes taken from two different hosts of the same species. That this difference in length is not due to methods of preparation causing the elongation of one set and the contraction of the other is evident when a comparison is made of the diameters of the body in the region of the nucleus. The long specimens from newt 19 were also thicker than the short specimens from newt 15, the former averaging 3.3$\mu$ in diameter, and the latter only 2.2$\mu$. The trypanosomes in newt 19 were therefore uniformly larger in all dimensions than those in newt 15.

Further study of Table 1 shows that the sets of 10 trypanosomes from each of the 10 newts were comparatively constant in their measurements. On the whole, the longest specimens are also the thickest, and length and width decrease together as one proceeds down the table.

In every set the average distance from the anterior end to the center of the nucleus is greater than that from the center of the nucleus to the kinetonucleus. When these distances are compared in the different sets, however, considerable variation becomes evident. For example, in specimens from newt 19 the difference between these two distances averages 10.7$\mu$ whereas in those from newt 17 the average difference is only 0.1$\mu$ and in those from newt 15 only 0.8$\mu$. These distances are more uniform also in trypanosomes from newt 15 than in those from the other newts. The variations in the distances from the kinetonucleus to the posterior end were slight in the trypanosomes of each set, but the average distance is greatest in those of the longest set and becomes gradually less as the total length decreases.

After the work just described was completed it seemed desirable to obtain a more accurate measure of the differences between the trypanosomes in newts 19 and 15. Ninety more specimens from each newt were therefore measured by my assistant and the results are indicated in Table 2; this gives the averages of the various distances for the first ten trypanosomes measured from newts 19 and 15, for the succeeding ninety and for the entire one hundred. It is interesting to note that the averages for the first ten are very nearly the same as for the succeeding ninety. This indicates that the data obtained by measuring ten specimens from each newt as presented in Table 1 give fairly accurate averages.

Among the specimens from newt 15 six were found that were very much larger than any of the others. Measurements of these six are given in Table 2 and were omitted from those used for getting the averages of the 100 specimens given in this table. These six specimens resemble very closely those taken from newt 19 and apparently represent a type differing widely from the other more abundant trypanosomes from newt 15. Several explanations suggest themselves to account for the diversity between these two types found in a single host. Most probably there are here two size races of one species or there may be two distinct species living in a single host. The two types may possibly represent sexual stages of one species and although from what is known of the life cycles of other blood-inhabiting protozoa one would expect to find the sexual stages in the invertebrate host, still, as in the malarial organism, gametocytes may be developed in the vertebrate and remain dormant until stimulated to further activity within the invertebrate host. It is also possible that the two

types of trypanosomes from a single host may be due to different stages of growth. A final suggestion is that *T. diemyctyli* is dimorphic.

## DISCUSSION

No one has succeeded in classifying satisfactorily the trypanosomes and their allies, a condition due in part to the difficulty of determining morphologic differences of diagnostic value and to the fact that many species are polymorphic. *T. diemyctyli* is a favorable form for study because it is apparently monomorphic. Its life history, however, is unknown. The account Tobey gives of this species and the experience of the writer indicate that types other than the long, slender form do not occur in the blood of the newt, at least at the time of year when the examinations were made (May). No specimens were found that showed any signs of division and hence it seems safe to assume that all of the organisms measured represented "adult" forms.

TABLE 2.—AVERAGE LENGTH AND WIDTH IN MICRONS OF SPECIMENS OF *T. diemyctyli* FROM NEWTS 19 AND 15

| | Anterior End of Body to Center of Nucleus | Center of Nucleus to Kineto-nucleus | Kineto-nucleus to Posterior End | Total Length Exclusive of Flagellum | Width of Body at Nucleus |
|---|---|---|---|---|---|
| Specimens from newt 19: | | | | | |
| Average of specimens 1-10..... | 36.3 | 25.6 | 6.1 | 68.0 | 3.3 |
| Average of specimens 11-100... | 35.1 | 28.0 | 6.5 | 69.6 | 3.6 |
| Average of specimens 1-100.... | 35.7 | 26.8 | 6.3 | 68.8 | 3.5 |
| Specimens from newt 15: | | | | | |
| Average of specimens 1-10..... | 21.4 | 20.6 | 2.7 | 44.7 | 2.2 |
| Average of specimens 11-100... | 21.9 | 21.4 | 2.3 | 45.7 | 2.9 |
| Average of specimens 1-100.... | 21.7 | 21.0 | 2.5 | 45.2 | 2.6 |
| Average of 6 largest specimens | 33.5 | 26.3 | 4.8 | 64.6 | 3.5 |

Two hypotheses suggest themselves to account for the constant diversities in the total length and the length of portions of the trypanosomes from the different individual newts; (1) the observations may deal with pure lines, and (2) the organisms in one newt may be derived from various lines but may have become comparatively uniform in size due to life in one environment. The differences between groups of trypanosomes from different hosts might be accounted for by differences in the environment.

*Pure Lines in Protozoa.*—It has been shown by many investigators that "wild" specimens of free-living protozoa differ from one another in their heritable characteristics and that the descendants derived by vegetative reproduction from one "wild" individual may be uniformly different from those descended from another "wild" individual. The number of these pure lines that may exist in nature seems almost infinite.

Considerable interest has recently been created by the discovery of different strains of cysts of *Entamoeba histolytica* and *E. coli*. Mathis and Mecier (1916, 1917) recognize cysts of two sizes from *E. histolytica* which they consider indicates a sort of sexual dimorphism. Various strains of cysts as regards size have also been noted in *E. histolytica* by Wenyon and O'Connor (1917), Dobell and Jepps (1917), Matthews (1918), Mackinnon (1918), Smith (1918, 1919) and Kofoid, Kornhauser, and Swezy (1919). The evidence indicates the existence in these parasitic protozoa of heritably diverse races similar to those that have been described in a number of free-living protozoa. What influence environmental factors may have on the size of the cysts can be determined in several ways; for example, single specimens could be isolated from cultures and pure lines obtained from these also in culture. The effects of changes in environment could then be observed by modifying the culture medium or by inoculating specimens from the same pure line into different laboratory animals.

The habitat of the intestinal amoebae resembles that of the trypanosomes in certain respects although it may be more or less varied because of the many different kinds of food taken into the alimentary canal. The composition of the blood differs in different species of animals and to a lesser degree in different individuals of the same species. This means that trypanosomes also are subjected to differences in their environment. No one knows what effect these different environments may have on trypanosomes belonging to the same pure line, but as noted above a method of determining this point is available and is being put to the test in this laboratory.

## SUMMARY

(1) Every one of 78 aquatic specimens and 2 of 7 land specimens of the newt, *Diemyctylus viridescens*, collected in Pennsylvania were found to be infected with *Trypanosoma diemyctyli* Tobey. No trypanosomes were found in 72 specimens of 5 other species of salamanders. Inoculation experiments with *T. diemyctyli* on 6 species of salamanders and 2 species of frogs were unsuccessful.

(2) Measurements were made of trypanosomes from 10 newts. These groups of trypanosomes differed from one another in their range of variation in total length exclusive of the flagellum, in the length of portions, and in the width of the body, in the average length of the entire body exclusive of the flagellum, in the average length of portions and in the average width.

(3) Length and width show a positive correlation and on an average the longer the specimen the wider it is.

(4) Of 106 trypanosomes from one newt, 100 were uniformly small and the remaining 6 were much larger, indicating 2 different types in a single host.

(5) The different types of trypanosomes obtained from the different newts are probably races of one species that are heritably diverse in size. They may, however, belong to different species or may be sexual phases of a single species, or may differ because of changes due to the environment.

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References to other literature on the specific identification of trypanosomes may be found in the above papers, especially that by Pearson.

# A NEW BLOOD FLUKE FROM TURTLES*

HENRY B. WARD

For several years an interesting trematode has been under observation in the laboratory here. It occurs in various species of turtles, and was first discovered in some material shipped in from the south for class work. Peculiar importance attaches to the fact that it is a species inhabiting the circulatory system, and in fact it shows a relationship to the blood-inhabiting flukes of man which has become more clearly evident as the observations have accumulated. Since the material is easily obtained, it will afford perhaps the best opportunity available in this country for the laboratory study of forms adapted to this peculiar environment, so that, despite the incompleteness of the observations, the publication of this note is justified. It is further called for by the fact that several others, who had their attention called to this species, plan to give it a more detailed study than I can make at the present time, and will be glad to have a record of the facts thus far determined in order to utilize them as a basis for further study.

For this very unique species I propose the name *Proparorchis artericola* gen. et spec. nov.

The parasite has been found in several distinct species of turtle from widely separate localities. Thus, according to records of the collection here, it has been met with in *Pseudemys elegans* from Havana, Illinois, in *Malacoclemmys leseuerii* from Newton, Texas, in *Pseudemys scripta* from Raleigh, N. C., and in *Chrysemys marginata* from Fairport, Iowa.

## OBSERVATIONS ON LIVING MATERIAL

The general distribution of the parasite in the body of the host is well illustrated by the record of one very careful examination made in May, 1915. The specimen was *Pseudemys scripta*. The circulatory system was first studied and the examination of a large quantity of blood gave only negative results. After ligating veins and arteries, the heart was removed and four flukes found in it. Several large veins were taken out and teased, but no flukes obtained. When, however, the large arteries were subjected to similar treatment, three flukes were taken; one was found plugging up the end of an artery. Both lungs were teased out; one yielded three flukes, the other none. Negative results came from similar handling of the liver. All organs examined

* Contributions from the Zoological Laboratory of the University of Illinois, No. 176.

contained eggs. A large number were present in the brain. They seemed to be more numerous in the lungs and digestive system than in the muscles.

A year later another turtle of the same species was examined with almost identical results. Every precaution was taken to prevent transfer of material from one organ to another or loss of any during the examination. The heart contained four large flukes, the arteries three, and the veins none. One lung yielded three flukes and the other none. The intestine and mesentery were stretched out and fixed in that condition. A methodical examination revealed no flukes in these organs altho eggs were very abundant in the mesenteric vessels.

When the living worms are released from blood vessels into normal salt solution, they often display marked activity and swim about so rapidly that it is difficult to follow them. The method of progression in the fluid resembles that of leeches and is sufficiently powerful to convince the observer that they can probably make progress against the blood stream in the arterial circulation. Their orientation in respect to the direction of the current in the vessels varies.

As has already been noted, they were never found in the venous circulation, but were taken in arteries and in the heart. This location was finally verified by ligating the blood vessels, removing the heart and sectioning it *in toto*. A fluke was found in the ventricle. In this host, also, it was found that eggs were very abundant in the walls of the ventricle and present tho less abundant in the walls of the auricles. The numerical difference was somewhat proportional to the thickness of the muscular tissue in these two regions. The turtle examined in this instance was rather small, and hence young. Yet it was generally infected with eggs in various tissues.

Almost all tissues contain eggs in case the turtle is generally infected but the mesentery and the lungs seem to accumulate the most. In extreme cases these organs are crowded so full that, as can be most easily observed in the case of the mesentery, the eggs serve to outline the course of the arteries. In the mesentery the ova vary widely in color and general appearance. Some are only faintly colored with a clear transparent shell that enables one to determine readily the character of the enclosed embryo. Those at the other extreme are deep brown, almost black, and entirely opaque. None of the shells had opened and apparently none of them escape from the vessels. The mesenteric arteries would thus form a graveyard in which ova would accumulate without achieving for the species their purpose.

It is interesting to compare with this an observation reported to me by one of my students. He examined a small turtle that had died after being kept some time in a laboratory tank and found the lungs filled

with eggs. Many of them were empty shells from which the miracidia had escaped for the lid was open. It is of little value to speculate on the fate of these embryos. It is clear, however, that if at the time of oviposition the flukes resort to the ventricle, which they surely do visit as I have shown, then the ova will be carried in part into the systemic arteries and in part into the pulmonary arteries. The ultimate results seem to be different in the two cases. Eggs have also been obtained from the feces of infected turtles.

The number of parasites found in a single host has never been very large whereas the number of eggs was often very great. This points to the gradual accumulation of the ova in the vessels, perhaps over a considerable period. Some turtles have been examined without finding any of these flukes and yet eggs occurred abundantly in the tissues. In other cases only very young flukes were found and these had not yet begun to produce eggs altho the eggs were numerous in the mesenteric vessels. While some parasites might easily be overlooked, yet these cases indicate that the flukes which had produced the eggs, had died and the young parasites were a later infection.

Some observations were made on the eggs containing living embryos. For this purpose eggs were taken from the feces of *Chrysemys marginata* and treated with dilute solutions of HCl from 0.02 to 0.1% in strength. The effect on the miracidia was very evidently stimulating, but those that hatched out were killed by the action of the acid immediately after the rupture of the membranes, and some were killed while even yet within the unbroken egg shell. This would seem to indicate extreme sensitiveness to gastric digestion and preclude direct infection of a new host by way of the alimentary system at least.

Eggs placed in hanging drop cultures hatched out in from 4 to 24 hours after being mounted. From these eggs and also from others placed in pure water, the miracidia seemed to emerge sooner than from eggs left in feces in a petri dish. When eggs with mature embryos are broken open in normal (0.75%) salt solution, the miracidia can be studied and later preserved. Free miracidia were found in feces cultures, but all those seen there were dead. Their escape from the shell was not observed. Other eggs in the feces contained apparently fully developed miracidia that had died without hatching out. No light was obtained on the conditions controlling the normal escape of these embryos from the eggs.

The unripe embryo is rather quiet, but shortly before hatching it becomes increasingly active, first by moving parts of the body and then by rotation on its longitudinal axis. This rotation increases in rapidity and is accompanied by pronounced contractions of longitudinal and circular muscles which turn the embryo so that it may assume any posi-

tion within the shell. These violent movements ultimately loosen the cap of the egg shell and tilt it to one side like a lid fastened by a hinge. The miracidium forces its way bit by bit thru the open door which is not large enough to permit its immediate exit, but once free it swims round and round in the free water with relatively great speed and energy. In all observations made here, they lived only a very short time (5 to 10 minutes), becoming rapidly distorted and ceasing all activity thereupon. It is probable accordingly that these observations did not present normal conditions for opening the eggs or for the miracidia afterwards. Of course the conditions may have brought about precocious hatching, but none of the eggs hatched which were held under observation for longer periods.

Miracidia still enclosed in the egg shell measure about 28 to 14$\mu$ whereas those free in water or feces are distinctly longer and slenderer, measuring about 30 by 11$\mu$. Within the shell one finds a large oval globule (Fig. 7) slightly greater in dimensions than the embryo. The miracidium has a large black eye spot which always appears irregular, and in favorable circumstances shows the form of contiguous reversed crescents usually designated as X-shaped. Some large gland cells are seen faintly in the living specimen, and its surface is covered with a coating of long cilia which are comparatively thinly distributed. The anterior end carries a cap-like structure which in the free swimming miracidium (Fig. 9) becomes a small bluntly rounded conical papilla. The ducts of the glands open on the summit of this papilla.

### STRUCTURE OF THE ADULT PARASITE

The adult worms, which are easily found on careful examination of the mesenteric vessels of infected turtles, are small and conspicuously transparent. In size, they measure from 1.62 by 0.28 to 2.62 by 0.77 mm. In the smallest the ovary was small and no ovum had yet developed but ripe sperm cells were found in testes and vesicle. The body is an elongated oval, or spindle shaped tapering slightly towards sharply rounded ends. The anterior end is more nearly pointed and much more mobile than the posterior. The body is relatively thin, measuring not more than 70 to 80$\mu$ in dorso-ventral diameter, and in the preserved specimen is regularly hollowed out a little on the ventral surface both longitudinally and transversely. The margins of the body are noticeably thin and sharp. In the blood vessels the worm appears to be much slenderer and longer than when observed outside the body of the host or in alcoholic specimens. The transparency of the body is due to the relatively slight development of the muscular layers which are represented only by thin sheets of very delicate fibers.

At the anterior end one notes the single sucker present in this species. It is peculiar in form, being a greatly elongated oval with relatively small sub-terminal opening. It projects forward in an unusual fashion, and imparts to the anterior end a characteristic appearance which is rarely met with among trematodes.

The surface of the body is smooth and without spines or scales. None of the small wart-like structures with fine spines have been found in this species which are described by Looss and others for other types of blood-inhabiting flukes. In preserved worms, which are somewhat contracted at the anterior end, the esophagus is slightly sinuous and the inner wall plicated. It has a relatively large lumen and increases in external diameter posteriad. The cavity varies noticeably in width, having one or two wider regions much such as are figured in the Schistosomatidae by Looss (1895, pl. 2, fig. 18). There is no evidence whatever of a pharynx, but near the posterior end (Fig. 5) the wall of the esophagus is conspicuously thickened by an accumulation of what are certainly gland cells. These take a deep stain, and while so irregular in form as to preclude the possibility of interpreting them as a muscular organ, yet superficial examination might lead one to designate this region as a pharynx. It is, however, at the very termination of the esophagus, taking in one-fifth or one-fourth of the entire length of the organ and not separated by any interval whatever from the diverging crura. These gland cells are densely crowded and in this posterior region occur in several layers so that they seem to form an enlargement of the esophageal wall. A thinner layer covers the wall of the esophagus for its entire length. Similar conditions were originally described by Leuckart for Schistosomes and fully verified by Looss. The likeness between Proparorchis and Schistosoma in respect to the esophagus is so complete that it extends even to minute details of structure. Looss (1899:751) reported similar glandular structures in Hapalotrema and denominated them salivary glands. It is thus evident that they are all but universal in blood-inhabiting flukes and indeed will probably be found in species from which they have not yet been recorded. Their development is undoubtedly due to the type of food utilized by these flukes.

The intestinal crura are markedly sinuous in outline and nearly equal in caliber throughout the entire length. They extend to within a short distance of the posterior end and there turn somewhat towards the center, although always remaining distinctly separated from each other. The cells which line them are filled with a dark granular substance, suggesting the origin of this material from the blood of the host (Cf. Looss, 1895, pl. 3, for Schistosomes). The crura diverge almost at right angles from the esophagus, forming a conspicuous cross bar

and an equally conspicuous angle at the side where they turn backwards. Directly opposite the point of junction with the esophagus is a median structure which stains conspicuously and is apparently glandular. It has only in part the same appearance as the crura themselves, and might be regarded as a median diverticulum with a very short lumen. However, the cells are not filled with the dark granules which impart to the intestine its characteristic appearance. The lining of the diverticulum is a very thin membrane and at its base is a mass of amorphous material which resembles in appearance and in staining qualities the inner layer of the esophagus (Fig. 5).

The excretory system is easily seen at the posterior end of the body. It presents the form of a bifid bladder or perhaps of small lateral bladders connected by very short stalks to a common duct, which is equally brief and opens at the median pore. The latter is nearly terminal in location. The lateral bladders are a little shorter than the space between the posterior tip of the body and the end of the intestinal crura. Anteriorly one sees a single longitudinal vessel connected with each bladder. Further details of the system have not been worked out.

The main features of the nervous system are distinct in living specimens and also in toto-preparations. The anterior ganglion spans the esophagus a short distance back of its junction with the oral sucker. The lateral nerves are relatively heavy and can be traced the length of the body. These features are relatively larger and more conspicuous than in most flukes. Here again the conditions recall those in the Schistosomes as reported by Looss (1895).

The most striking feature in this parasite is the peculiar development of the reproductive system. The organs are nearly all confined to the area within the intestinal crura. The testes (Fig. 1, *t*) occupy the major portion of the space anteriorly. They begin a short distance behind the fork of the intestine and extend as a series of irregularly lobed bodies down the median line a distance equal to about one-half of the entire length of the worm. In this group are from six to ten or more irregularly shaped bodies, more or less flattened on the anterior and posterior faces by mutual pressure but deeply lobed on the lateral aspects. In many cases it looks as if the parts were continuous, but sections show well developed limiting membranes separating them. It may be that there is a fixed number of separate parts in this testicular area but the varying stages of contraction in different specimens make it difficult to reach a positive conclusion. Immediately behind the posterior testis is a seminal vesicle (Fig. 3, *sv*) which is elongated, pyriform and connects directly with the cirrus (*c*). No distinct prostate cells were seen and both the cirrus and the cirrus sac are delicate and difficult to detect. The pyriform vesicle and the duct form a nearly

straight passageway from the center of the posterior testis to the common genital pore (*gp*). This opening is located about on the level of the intestine at the left side and ventral.

The ovary (*ov*) is a many-lobed structure in the intracrural area behind the testes; it lies chiefly dorsal. The vesicle and cirrus cross ventrally the left ovarian lobes. The opposite face of the organ is pressed closely against the intestine on the other side of the body. The yolk glands (Fig. 3, *v*) are exceedingly voluminous. They begin about at the end of the esophagus and extend just a little beyond the posterior ends of the intestinal crura. The cells tho not crowded form an almost continuous strip or band which lies below and, to some extent, on both sides of the crura but only in the immediate proximity of those structures, for the central area of the body is entirely without yolk cells. At the end of the esophagus and behind the crura, the cells from the two sides approach and become confluent in the median line. Behind the ovary on the ventral side of the body, the transverse yolk duct joins the two yolk glands and on it in the median line is formed a prominent yolk reservoir (*yr*). There are no reproductive organs behind this limit, except some of the outlying cells of the vitellaria already mentioned.

The ducts of the female system are strikingly simple, and are crowded together in a small triangle between the ovary, the cirrus, and the transverse yolk duct. The relation of the different structures will be apparent from the illustrations (Figs. 2, 3) one of which represents a reconstruction of this area from a series of sections. One can readily identify the various structures. A small expansion on the oviduct near the ovary is seen to be the receptaculum seminis uterinum (*rsu*) which is easily recognized by the considerable mass of sperm cells that it contains. After a brief course dextrad and posteriad the oviduct turns sharply back on itself near the intestine and swings in a crescentic curve to the sexual pore on the opposite side of the body. About at the angle made by this turn, there is given off a short tube which mounts almost directly to the dorsal surface. This is Laurer's canal (*lc*). It is relatively large and open, and contained ripe sperm cells in those specimens which were sectioned. Half way from this point to the genital pore, the canal is slightly expanded, and in this expansion lies in many specimens a single egg. This tube corresponds in position and connections to the long convoluted uterus in most flukes, but instead of carrying a mass of eggs such as is usually found in that organ, it never contains in this form more than a single one. The short stretch which intervenes between this region and the pore is distinctly provided with a muscular layer in the wall (Fig. 4). This region is the metraterm; the egg lies really in the ootype and a true uterus is lacking.

The eggs outside of the worm in the blood-vessels of the mesentery as already noted are in part light colored and semi-transparent, and in part dark brown and almost opaque. The latter seem to be the older eggs. Sets of eggs from different places were measured, and the results are given in the following summary. All measurements are given in microns.

| Preparation, No. | Number Measured | Average Length | Average Breadth | Length Max. | Length Min. | Breadth Max. | Breadth Min. |
|---|---|---|---|---|---|---|---|
| 15.54 | 19 | 95.9 | 75.5 | 105.6 | 70.4 | 96.8 | 61.8 |
| 15.71 | 20 | 103.4 | 77.4 | 123.2 | 88.0 | 88.0 | 70.4 |
| 15.71 | 20 | 106.5 | 78.7 | 124.2 | 81.0 | 94.5 | 64.8 |
| 15.72 | 20 | 110.4 | 81.7 | 121.5 | 97.2 | 91.8 | 75.6 |
| 15.72 *l* | 12 | 101.2 | 82.7 | 114.4 | 70.4 | 96.8 | 52.8 |
| 15.72 *d* | 20 | 95.9 | 77.0 | 114.4 | 79.2 | 88.0 | 52.8 |
| General average..... | | 102.2 | 78.8 | | | | |

*l* = light eggs only; *d* = dark eggs.

Most eggs come within the limits of 88 to 114$\mu$ in length and 70 to 88$\mu$ in breadth.

When these figures, which are obtained from preserved material, are compared with those giving dimensions of the eggs under other conditions, the results are rather extraordinary. In living specimens eggs from cultures, drawn and measured were 85x68 $\mu$, 97x80 $\mu$, 80.5x73 $\mu$, 85x74 $\mu$, 97x80 $\mu$. In worms which had been preserved, stained and mounted *in toto*, the eggs still contained in the uterus of the female showed dimensions of 75x45 $\mu$ and 84x41 $\mu$. The eggs from cultures and especially those still retained in the body of the female are thus smaller than the average of those found free in the blood-vessels of the mesentery. Further, eggs in the body of the worm are clearly oval, whereas those outside are more nearly spherical. Looss (1902:522) noted also that the egg shell may increase in size during the growth of the embryo; this was observed in a blood-inhabiting species the adult of which was not identified.

The eggs found in various organs occasionally show stages in cleavage or much more often two black eye spots indicating that the miracidia are well developed. One encounters also many eggs in which the enclosed embryos are dead and undergoing disintegration. One of my assistants at one time found a mass of eggs in the intestine of a turtle but no evidence was secured on the method by which the eggs escaped from the vascular system or the place at which such escape was made; and the discovery noted may have been based on some sort of accidental transfer of the eggs to the intestinal contents. Nevertheless there is no doubt that eggs occur regularly in the feces of the turtles for they have been collected and studied frequently and those found there contain living embryos.

The data just given on the structure of the parasite may be summarized in the form of a generic description as follows:

PROPARORCHIS Nov. Gen.

Small trematodes with delicate body, widest at center and tapering towards both ends. Oral sucker elongate, protruding; no other sucker present. Esophagus with wide lumen, without pharynx, covered with glandular cells, prominent near posterior end; crura long, sinuous, extending nearly entire length of body. Median glandular diverticulum opposite end of esophagus. Excretory bladder double, short; excretory pore single, subterminal. Genital pore sinistral, ventral, in posterior region. Cirrus sac with slender cirrus and ductus ejaculatorius; seminal vesicle large, pyriform. Testes numerous, irregular, lobed, in intercrural area, between intestinal fork and ovarian complex. Ovary lobed, posterior to testes, chiefly on right side of body; oviduct short, with sperm filled expansion (receptaculum seminis uterinum). Laurer's canal present but no receptaculum seminis. Vitellaria well developed, conspicuous laterally, enveloping intestinal crura on lateral, dorsal and median aspects thruout entire length. Transverse yolk duct with median reservoir between ovary and end of crura. No true uterus present, metraterm extends straight from ootype to pore. Eggs deposited so soon after formation that never more than a single one is seen in the fluke. Egg provided with cap; those in body of worm measure about 80 by 45 $\mu$, in blood vessels of host about 100 by 80 $\mu$. Cleavage well advanced before oviposition; well developed miracidia with conspicuous eye spots in eggs taken from blood vessels of host.

Type and only species: *Proparorchis artericola* from various fresh water turtles.

The data in my possession are not all referable to the single species which has just been described. In details of structure, in regard to the eggs, in the location in the host in which they have been observed, and in some other details, certain specimens differ so distinctly from the account above that I can not at present include them under the same heading. It is possible that they represent different phases in the life cycle of a single species. I am inclined to think the structure of this worm too delicate for one to consider it probable that any part of its life history could be passed in the intestine. But such a transfer must still be kept in mind as a possibility. In my opinion it is much more likely that further study will disclose the presence of several species parasitic in the blood of reptiles and amphibia. I have myself a single specimen of a distome unlike any genus yet described which was found in the course of explorations for the species just described.

## RELATED SPECIES OF FLUKES

In a recent paper (G. A. MacCallum, 1919) has given a brief description of an unusual worm found in the *intestine* of a wood turtle at the New York Aquarium. This form is undoubtedly closely related to that described in this paper. To this form MacCallum gave the generic name, *Spirorchis,* but omitted to add any specific designation. In order to insure accuracy of reference, I would suggest that his species be designated *Spirorchis innominata.* MacCallum's description is brief and in some details confused since the dimensions given are clearly wrong and the text does not agree in full with the illustration. On the other hand no one can consider his account without being impressed by the general likeness his species has to that described here. It is important to consider in a comparative fashion the structure of these two forms as a basis for a decision as to the degree of their relationship.

MacCallum's fluke is considerably larger tho of the same general form and apparently also similarly delicate in structure and transparent. The general plan of the organs is much like that in the species just described. The peculiar form and position of the oral sucker in Proparorchis is both described in MacCallum's text and shown in the figure accompanying it; on the other hand, the characteristic angle of the esophagus and crura in this form is not shown or mentioned by MacCallum, and the pharnyx which he describes and pictures near the center of the esophagus is certainly not present at all in the blood fluke I have studied. Many further items in MacCallum's account, like the appearance of the testes and of the seminal vesicle, the size and form of the ovary and of the egg, and the various measurements of the body which do not agree with the description given above, may perhaps be explained as specific differences tho they are not discussed in sufficient detail to make this opinion positive. His very definite statement concerning the location of the genital pore shows a striking difference from the condition in the species described here.

MacCallum states that his parasites were taken from the intestine of a wood turtle *(Chelopus insculptus)* but adds "as will be seen by the color of the contents of its intestines, it is a hematophagic trematode." It is not unlikely that its presence in the intestine was accidental, the result of opening some blood vessel during the dissection, and that in fact that species also is normally an inhabitant of the vascular system. But this is only a tentative opinion.

The converse of that proposition is entirely untenable. One can not maintain the view that the worms I found in the blood vessels are immature forms which might later attain the adult condition in some other location. Several conditions militate against such an explanation. First, large masses of ripe sperm cells are found in the worms, and

occur not only in the seminal vesicle, but also in that portion of the oviduct often termed the receptaculum seminis uterinum. This shows that impregnation has already taken place altho, of course, this might have been self-impregnation which has been observed in encysted trematodes. Secondly, many if not all of the flukes contained in the uterus a single egg which was well started in development. Third, careful microscopic inspection of the ovary and testes gave evidence that these organs in some cases had been functioning for some time. Fourth, the blood vessels contained large numbers of egg shells which enclosed fully developed embryos; these were removed from the vessels and watched in many instances until the miracidia hatched out and swam about in the culture medium. Fifth, all stages in advancing maturity which should be present in adult worms are actually represented in the specimens found, from the young fluke in which the female organs have not yet begun to function actively, to such as show that system at its functional apex whereas the male organs have passed their prime and are already on the decline. Proparorchis becomes fully mature in the blood vessels of the host.

I have endeavored as yet unsuccessfully to get for examination one of the specimens on which MacCallum's description is based. In the light of his description it appears to me necessary to accept his diagnosis as it stands, especially since his previous publications show great care in studying out similar parasites and accuracy in stating the results of such study. Unless the differences are to be explained away as errors in observation, they form an adequate basis for the differentiation of genera even tho these are closely related and should be included in a single subfamily. I have rewritten MacCallum's account in brief taxanomic form in order to facilitate the comparison of that species with the one I have just described.

### GENUS SPIRORCHIS G. A. MAC CALLUM

Small species with smooth skin, body widest near center, tapering towards both ends. Anterior sucker small, protruding; no acetabulum present. Esophagus with pharynx near the middle; crura conspicuous because of dark granular contents, extend to near posterior end, sometimes coalescing there. Excretory pore near posterior end. Genital pore median, posterior to tips of intestinal crura. Vitellaria profuse, lateral, along entire length of intestinal crura; transverse yolk duct and median reservoir near posterior limits of yolk glands. Ovary lateral, oviduct long; one large egg measuring 100 by 50 $\mu$,* with thick shell regularly present. Testes irregular, lobed, "in rough spiral column" occupying central region between intestinal crura and followed by large

* MacCallum says 5 to 10$\mu$, an evident error.

conical seminal vesicle which tapers to cirrus (?). Connection of ducts and other organs not seen "on account of black intestines filling the posterior end of the worm."

The genera Proparorchis and Spirorchis are evidently members of a new subfamily to which the name Proparorchiinae may be given. The position of this sub-family deserves further consideration. Its nearest affiliations are to be found on the one hand in a fluke from sea turtles described by Looss and on the other hand in the human blood flukes, the Schistosomatidae.

The first mentioned trematode from the vascular system is *Hapalotrema constrictum* (Leared), most recently studied by Looss (1902:519-). It is a common parasite of *Thalassochelys corticuta,* a sea turtle taken on the Egyptian coast. The eggs are very striking, being large and supplied with long polar processes coiled at the tip. They also occur within the tissues. While these eggs are so conspicuously unlike those of the species described in this paper, yet they enclose an embryo said by Looss to resemble closely that of Schistosoma, as also does the embryo of this species. In general appearance and structure, like Proparorchis, these worms are delicate, thin-skinned and only weakly provided with dermal musculature. In this respect they resemble also the human blood flukes (Schistosomatidae) most strikingly.

In discussing the genus Hapalotrema, Looss (1899:656) comments on its striking similarity to the genus Schistosoma in the alimentary system, the structure of the suckers and the character of the skin. Yet in view of the marked dissimilarity in other respects, he concludes that the likeness is merely convergence due to the place and mode of life since both inhabit the blood stream and feed on the blood. The resemblance to the species reported here is even more striking. The delicate body with scantily developed musculature, the peculiar esophagus, the short female genital canal, the formation and extrusion of eggs one by one, and the well developed ciliated miracidium are all peculiar and characteristic features in which the two forms agree with each other and differ from almost all other known trematodes. While a few of these features are found in the Schistosomes, as noted above and that agreement might be explained as the result of convergence, yet a similar explanation is more difficult to apply to the longer series of structural likenesses between Hapalotrema and Proparorchis.

However, the differences between the two species deserve equal emphasis. First of all, some would list the fact that Hapalotrema is a true distome whereas Proparorchis is a monostome; but to me this is a subsidiary feature since extended studies have led me to the full acceptance of the view presented forcibly by Odhner that the mono-

stomes do not constitute a natural group but represent an assemblage of forms derived from different families of distomes by the reduction and ultimate disappearance of the acetabulum. They are thus alike primarily only in the superficial feature that all possess but a single, anterior sucker. The organ pattern, on the other hand, brings evidence of marked differences in type and indicates clearly relationship to different groups of distomes. Odhner would accordingly classify the monostomes with those distomes to which they are most clearly allied and abolish entirely the major subdivision of Monostomata. For evident practical reasons, such a radical proposal is not likely to be adopted until knowledge of the monostomes has been greatly extended.

The oral sucker of Hapalotrema is described by Looss as flat, saucershaped, projecting above the general surface of the body and scantily developed in musculature. It appears thus as if already in course of elimination, even tho the process is only well begun and the interval that separates the species from Proparorchis is still great.

There are, however, still other and much more significant differences between the two species. In Hapalotrema, according to Looss, the excretory bladder is short and branches just behind the posterior testis.* This does not seem to be the condition in Proparorchis as described above. The genital pore in Hapalotrema lies about in the center of body in the left rather than as in Proparorchis near the posterior end. The eggs are very dissimilar in general appearance, since those of Hapalotrema are provided with two long polar processes on the shell which are spirally twisted at the end. It may be noted that this difference is of the same sort that exists between *Schistosoma haematobium* and *S. japonicum,* tho of course more extremely developed. The egg here has a cover and no such structure is shown for Looss' species.

By far the most striking difference is one that affects the general appearance of the body very radically. At first glance Hapalotrema resembles a common type of distomes; the transverse yolk duct, ovary, seminal vesicle and cirrus lie in the central area and other genitalia are grouped symmetrically about them. In Proparorchis the genital complex lies near the posterior end and the other genitalia lie almost entirely in front of it. A closer analysis shows that Hapalotrema is really of an unusual type morphologically; the two testes are fragmented instead of simple, and one fills the central area anterior to the ovarian complex whereas the other testis occupies the corresponding posterior area.

Conditions in Proparorchis as described above permit of a close comparison with those in Hapalotrema if the posterior region in the latter be reduced by failure of the posterior testis to develop and coin-

* The text says *in front of* the posterior testis, but Looss' figure shows distinctly the other relation.

cident cessation of growth in the entire posterior half of the worm. As the result of this, the ovarian complex lies just in front of the posterior end of the body. The structures in this complex and anterior to it correspond very closely to those in the same regions in Hapalotrema.

Looss (1902) found in the blood vessels of sea turtles four types of eggs easily differentiated from those of Hapalotrema for which no adults were discovered despite the most careful search. These eggs, or at least two of the varieties, are much like those of Proparorchis.

## SYSTEMATIC POSITION

In a most interesting and suggestive paper on the Phylogeny of the Bilharzia type, Odhner (1912) established a new family, Harmostomidae, to include the subfamily Harmostominae already worked out by Looss (1899:651-) for a series of genera he had studied, and a second new sub-family Liolopinae in which Odhner includes Liolope Cohn 1902, Helicotrema n.g., and Hapalotrema Looss 1899. The Harmostomidae are placed close to the Schistosomes, which latter in the opinion of Odhner are "with absolute certainty" derived from the former. In my opinion the family Harmostomidae and the sub-family Liolopinae are both unnatural in one and the same particular: the inclusion of the genus Hapalotrema; for this form necessitates a series of exceptions in the descriptions that really do violence to the morphological basis on which those sub-divisions are built. Hapalotrema is unlike all other species in those two groups in the presence of a long esophagus, in the absence of a pharynx, in the form of the ovary and testes, in the absence of a true uterus, and finally in the character of the excretory system which is a most fundamental feature. Hapalotrema must be removed from Odhner's sub-family Liolopinae; it forms with the Proparorchiinae naturally a new family which may be characterized as follows:

## FAMILY PROPARORCHIIDAE

Delicate blood inhabiting flukes, with slender, non-muscular body and one or two weak suckers. Testes lobed, multiple, anterior (and sometimes also posterior) to ovarian complex. Laurer's canal present. Ovary lobed; no true uterus; eggs large, thick-shelled, discharged singly.

These forms are certainly related to the human blood flukes, Schistosomatidae, altho not so highly specialized for life in the circulatory system. Finally mention must be made of the peculiar blood-inhabiting fish parasites Aporocotyle and Sanguinicola which have been so thoroly studied and described by Odhner (1911.) These genera show evident morphological likeness to the forms discussed in this paper, and one is fully justified in associating relatively closely the families Aporocotylidae and Proparorchiidae.

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* Dated 1918 but not distributed until 1919.

## Explanation of Plate

*c*, cirrus; *e*, egg; *gp*, genital pore; *i*, intestine; *lc*, Laurer's canal; *od*, oviduct; *ov*, ovary; *rsu*, receptaculum seminis uterinum; *sv*, seminal vesicle; *t*, testis; *vi*, vitellaria; *yr*, yolk reservoir.

Fig. 1.—*Proparorchis artericola*. Toto mount viewed from ventral surface. Extent of vitellaria shown by dotted line. × 40.

Fig. 2.—Posterior end of same specimen. × 70.

Fig. 3.—Reproductive organs; reconstruction from sections. × 65.

Fig. 4.—Organs near genital pore, showing cirrus partly extended, metraterm, and egg just being formed. × 270.

Fig. 5.—Frontal section of esophagus, bifurcation of crura and diverticulum. × 170.

Fig. 6.—Egg in cleavage from ducts of fluke shown in Figure 1. × 210.

Fig. 7.—Miracidium, viewed in profile, in egg shell from culture; *x*, eye spot in dorsal aspect. × 360.

Fig. 8.—Empty egg shell, showing cap. × 360.

Fig. 9.—Miracidium just out of shell; ciliary coating omitted. × 150.

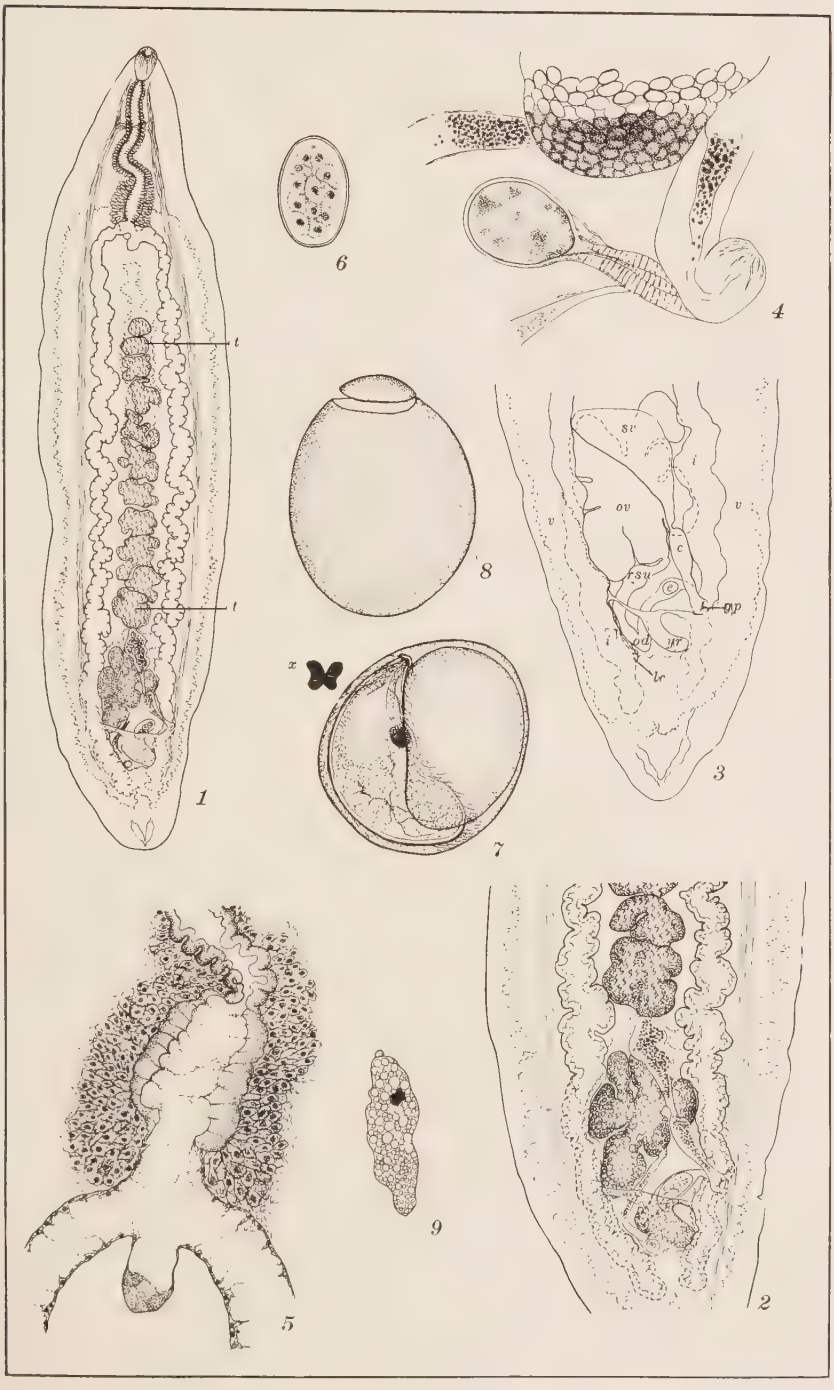


PLATE XII

# A NEW AMPHIBIAN CESTODE*

Lloyd B. Dickey

Few cestodes have ever been described from amphibian hosts. *Nematotaenia dispar* (Goeze 1782) has been reported from Europe by Schmidt (1885), and by Fuhrmann (1895); and from America by Stiles and Hassall (1912; 277). *Taenia pulchella* Leidy 1851 was reported from America in *Bufo americanus,* at the time of its original description. Jewell (1916) described *Cylindrotaenia americana* from America in *Acris gryllis, Rana pipiens, Rana virescens,* and *Bufo lentiginosus.* These three species, together with two Proteocephalids, *Ophiotaenia hylae* from Australia, and *O. schultzei* from Africa (Johnston, 1912), constitute the species so far described from frogs and toads. Johnston (1916: 194) reports the presence of a new species of Nematotaenia in Australia, which has not yet been described. Larval stages of Sparganum have been known to occur in European frogs, but nothing is known of the larval stages of any of the adult cestodes thus far reported in Anurans.

Jewell (1916) has pointed out the discrepancies of Schmidt's description of *Nematotaenia dispar* (Schmidt 1855) as compared with the recognized, original form first described by Goeze (1782), and later by Fuhrmann (1895). It is probable that Schmidt's material was not *Nematotaenia dispar,* since he describes the worm as having a neck, with the greatest diameter at the posterior end, and an oval cirrus sac about twice as long as it is broad. Fuhrmann's description includes a worm devoid of a neck, the greatest diameter of the strobila being at the anterior end, with the cirrus sac about ten times as long as broad.

Lühe (1899: 526) proposed the genus Nematotaenia, to contain *Taenia dispar* Goeze. His first characterization of the new genus, however, appears in a later paper (Lühe 1910), as follows: "Taenien mit unbewaffnetem Scolex, ohne Rostellem, mit drehrundem Körper, der in seinem vorderen Abschnitt etwas dicker ist und nach hinten allmählich dünner und schliesslich fadenförmig wird. Gliederung nur am Hinterende ausgesprochen, wo sich die reifen Proglottiden, die wesentlich länger als breit sind, einzeln ablösen, um dann lebhaft beweglich noch längere Zeit weiterzuleben. Gesclechtsöffnungen randständig, unregelmässig abwechselnd. Hoden in der Zweizahl, dorsal und annähernd symmetrisch. Dotterstock fast genau in der Achse

* Contributions from the Zoological Laboratory of the University of Illinois No. 177.

des Körpers; Keimstock ventral, der Genitalöffnung wenig genähert. Geschlechtswege dorsal von Wassergefässen und Marksträngen. Uterus frühzeitig in einzelne Eikapseln zerfallend, welche je 2-4 (meist 3) Eier enthalten. Eier mit 3 Hüllen. Finnenstadium unbekannt.

"Im Darm von Amphibien. Bisher nur eine Art bekannt."

Jewell (1916: 191) gives the following diagnosis for *Cylindrotaenia*: "Scolex unarmed, without rostellum; reproductive organs single in each proglottid; pores lateral, alternating; vagina and cirrus dorsal to the excretory canals and main nerve trunk; testis one, dorsal; ovary and vitellaria ventral. Uterus breaks up into capsules surrounding the embryos which ultimately pass into two parauterine capsules." *Cylindrotaenia americana* "from the small intestine of various Anura" is designated as the type.

## MORPHOLOGY OF THE NEW FORM

The present study is based upon material collected at Oxford, Georgia, in July 1916, from two host specimens of *Bufo lentiginosus*. Of the adult specimens, two bore ripe proglottids, and each measured about 100 mm. in length. Eleven young worms, which varied in length from 3.5 to 5 mm., were collected from a single host.

The worm is cylindrical in form anteriorly. In the region of the strobila where the reproductive organs attain the maximum of development, the segments are oval in cross-section, being compressed laterally (Fig. 1). In a typical specimen, the cross-section is circular in outline at a distance 6 mm. from the tip of the scolex, is oval at a distance of 13 mm., and the circular form is again assumed at a distance of 40 mm., in the region where the eggs have passed into the parauterine capsules and the uterus has broken up.

No external segmentation is apparent in the anterior region of the worm. It occurs rather distinctly from 48 to 60 mm. from the scolex. Here the segments begin to elongate and measure 0.24 mm. in length, and 0.39 mm. in diameter. The last few proglottids of the strobila measure 0.58 to 0.66 mm. in length and 0.18 to 0.24 mm. in diameter.

The scolex is unarmed, spherical, and broader than the neck. In adult specimens it measures 0.52 to 0.62 mm. in diameter. The neck averages 0.48 mm. in width. In young worms the diameter of the scolex is 0.26 to 0.33 mm. The suckers are situated near the tip of the scolex. They are unarmed, and have a diameter in the adult forms of from 0.093 to 0.14 mm. The diameter of the suckers in the young worms is from 0.085 to 0.09 mm. The lumen is directed anteriad and slightly laterad. The scolex is circular in cross-section, except through the region of the suckers, where it is slightly oval.

Two shallow grooves, extending from the tip of the scolex to the base of the suckers, occur on opposite sides of the scolex.

From the material at hand it appears that the cuticula is composed of two layers of equal thickness. The outer layer stains more heavily. The cuticula is 4 to 5$\mu$ in thickness and is supported by a delicate basement membrane. The subcuticula consists of cells 32$\mu$ long and 4$\mu$ in diameter. The nuclei are large and stain less heavily than the rest of the cells.

The well developed longitudinal muscles are arranged in a single layer. They separate the parenchyma into a cortical and a medullary area. The latter averages about 0.296 mm. in dorso-ventral diameter and about 0.222 mm. in lateral diameter in the region where the reproductive organs attain their maximum development. The longitudinal strands occur at approximately 0.083 mm. from the cuticula. About five or six small strands go to make up a large bundle. Between fifty and sixty of these bundles occur at more or less regular intervals. Many of them extend from one segment to another. Between subcuticula and cuticula the longitudinal fibers of the subcuticular muscles can barely be discerned. No trace of dorso-ventral muscles was found. The muscle strands of the longitudinal system are large and numerous, and are massed together at the tip of the scolex. There are also transverse muscles running concentric to the basement membrane of the suckers.

The ventral excretory canals are about 32$\mu$ in diameter. They pass lateral to the ovary about 50$\mu$ from the nearest longitudinal muscle strands. Commissures of the ventral excretory canals may be seen in the region where the reproductive organs first differentiate from the medullary parenchyma. These have a diameter of about 3$\mu$. The dorsal excretory canals are very small, and are seldom discernible. They appear most prominently in the region where the testes first become differentiated and here they often have a diameter of 8$\mu$. In the region of the scolex, they sometimes anastomose with the other excretory canals. The excretory system is continuous from one proglottid to another throughout the strobila. In the ripe proglottids the ventral canals present undulations, due to their position exterior to the developing parauterine capsules.

The two main lateral nerve trunks run parallel to and midway between the dorsal and ventral excretory vessels.

The genital rudiments are first seen at about 2 to 3 mm. from the tip of the scolex. They appear here merely as a dark streak running through the center of the proglottids. The testes are the first organs to become differentiated from the parenchyma. The ovary arises next, and the vitelline gland forms dorsad of the latter immediately afterwards. The testes are distinguishable 3 to 4 mm. from the sco-

lex. No external segmentation is apparent at this point. The internal or genital segmentation can be distinguished, however, the segments being 0.04 to 0.05 mm. long. All of the reproductive system, except the cirrus sac and vagina, is accommodated within the medullary parenchyma (Fig. 1). The genital pores are lateral and marginal, and alternate irregularly. The cirrus sac and vagina open into the genital atrium dorsal to the excretory canals and main nerve trunk.

The male organs are situated dorsally in the proglottid. The testes, two in number, are about 67$\mu$ in diameter at their greatest development. They are lenticular in shape and circular in cross-section, the antero-posterior thickness averaging 40$\mu$. This compression in the antero-posterior direction may be due to the contraction of the worm when killed, and the organs are probably spherical in shape in the live worm. A thin membrane surrounds each testis and is continuous with the walls of the vas deferens. The vasa efferentia, one from each testis, meet to form the vas deferens, near the testis on the poral side of the proglottid. After forming one or two short loops during its course, it passes into the cirrus sac (Fig. 1). The cirrus proper is surrounded by parenchymatous tissue composed of small cells with spherical nuclei. The cirrus pouch is flask shaped and is about one and a half times as long as it is broad. The length averages 48$\mu$, and the diameter 31$\mu$.

The ovary is a spherical organ, lying in the ventral half of the medullary region and slightly to the poral side of the proglottid. The diameter averages 67$\mu$. Numerous spherical cells, each enclosed in a capsule, make up the organ. The capsules average 12$\mu$ in diameter. The vitelline gland, also spherical in shape, lies dorso-lateral to the ovary but ventral to the genital pore. Its diameter averages 35$\mu$. The vitelline duct is directed laterad and meets the oviduct, which extends laterally and dorsally. The oviduct is continuous with the vagina, which in turn leads ventral to the cirrus pouch, running adjacent to it from the inner end of the latter.

The beginning of the uterus can be distinguished in proglottids 12 to 14 mm. from the scolex. The uterus is horseshoe shaped in appearance (Fig. 2). It arises from the medullary parenchyma and soon almost completely surrounds the vitelline gland. The ovary and testes break down at the same time, the ovary disappearing before the testes. At the maximum development of the uterus, about 18 to 20 mm. from the tips of the scolex, the eggs are 16$\mu$ in diameter. They are in the early stages of cleavage. The internal segments at this place are about 0.11 mm. long. The uterus breaks down early, about 22 to 24 mm. behind the scolex, and is replaced by the parauterine organs.

The parauterine organs arise from the parenchyma adjacent to the uterus. The strands of the meshwork of which they are com-

posed soon arrange themselves parallel to the uterus on the anterior side of the proglottid. The whole structure from this stage on grows very rapidly. The tissue migrates inwards, replacing the uterus by capsules, and surrounding the egg at the same time (Figs. 5, 6). The capsules are early seen to have well defined walls. All trace of the ovary disappears, but the remnants of the testes apparently persist as long as any trace of the uterus itself can be found.

From eight to twelve truncated or flask-shaped cones appear, arranged in two parallel rows (Figs. 3, 4), one row dorsal, the other ventral. There are from four to six capsules in each row, their usual number being five. A sort of raphe, which is composed of numerous, small, spherical and thickly massed cells, staining a dark gray with hematoxylin, separates the capsules of the two rows as they come together in the center of the proglottid. The basal portion of the capsules is in the posterior portion of the proglottid and the longitudinal axes of the cones correspond to the longitudinal axis of the worm. A meshwork of fine fibers together with a fine granular tissue adjoins the capsules in the basal portion. The apex, capped in each instance by the darkly-stained cell-gland secretion, is situated in the anterior portion of the proglottid. The minute cells, from which the secretion is emitted, are easily distinguished at the most anterior end of the apparatus. The length of the cones increases as the proglottids become elongated. At the time the cones attain their greatest length, a distance of 60 to 70 mm. from the head, they measure approximately 0.11 mm. in length. The width of the base of a cone averages 0.06 mm. in lateral diameter, and 0.09 mm. in dorso-ventral diameter. The capped secretion averages 0.3 mm. in width and 0.07 mm. dorso-ventrally.

The eggs, occupying the basal portion of a cone, are three to six, usually four, in each capsule. They are oval, averaging 43$\mu$ in length, and 31$\mu$ in diameter. The embryos are still in the spherical stage, and average 19$\mu$ in diameter. The eggs have a shell about 3$\mu$ in thickness. Only a single thin membrane could be distinguished surrounding the embryo.

As the proglottid elongates the cones change in position and shape (Fig. 7). By the time the proglottids have become detached, the cones have become more spherical, especially in the apical portions, which now appear larger than the basal regions. This growth takes place at the expense of the surrounding parenchyma, the latter furnishing the nourishment necessary for the growth of the capsule. The fact that the parenchyma is much less dense at this stage than in earlier stages is easily seen in cross-sections of the proglottids. Some of the eggs migrate from the base of the cone into the apex. The bases of the cones remain clustered together, while the apical

portions spread apart from each other irregularly in all directions. This is caused by the total disappearance of the raphe composed of the numerous small, spherical cells, which heretofore held the capsules together in the two parallel rows. The capped secretion spreads out over the apex of each capsule. Portions of the fibrous and granular tissue at the bases of the cones may become broken off and migrate into the parenchyma. This arrangement may be distinctly seen in detached proglottids.

Since the living parasites were not obtained nothing is known of their further development.

## COMPARISON OF FORMS

The form under consideration bears striking resemblances to *Nematotaenia dispar* (Goeze 1782). It is similar in external form and in the limitation of marked segmentation to the posterior end. The reproductive organs are similar in shape and position. There are two testes, and the ovary, vitellaria, and uterus are single in both cases. A difference occurs in the shape of the cirrus pouch. In *Nematotaenia* this is tubular, and about ten times as long as broad, while in the present form the cirrus pouch is flask-shaped, and about one and a half times as long as it is broad. The uterus in both forms is horseshoe-shaped, and breaks down early. The most marked differences between the two forms lie in the development, position, and number of the parauterine organs. In Nematotaenia there are developed a varying number of small parauterine organs. Ripe proglottids show from thirteen to thirty capsules, scattered irregularly throughout the parenchyma. In the new form, the number of parauterine organs is limited, the mature proglottids showing eight to twelve capsules, arranged in the two parallel rows.

The only description extant of *Taenia pulchella* Leidy 1851, is much too meager to permit of a satisfactory comparison with other forms. It has general similarities with the form under consideration, such as its occurrence in the same host genus and its external appearance.

Certain marked similarities occur between the new species and *Cylindrotaenia americana* Jewell 1916. In Cylindrotaenia the cylindrical form also occurs, and the segmentation of the strobila is evident at the posterior end only. The musculature of the two species is very similar. While in both forms the male reproductive organs are limited and definite in number, in Cylindrotaenia there is only one, and in the new form two, testes. In the former the vas deferens leads straight to the cirrus, while in the new worm there are various undulations. The female reproductive organs are very similar.

Again, the most marked difference between the two species occurs in the number and position of the parauterine capsules. In Cylindrotaenia two truncated cones appear, one dorsal and one ventral, the parauterine capsules being thus definitely limited. In the form here described, the capsules are also limited, although not as definitely, and they are more numerous than in Cylindrotaenia, being eight to twelve in number, with the regular arrangement previously noted.

## SYSTEMATIC POSITION

In the Revision of the Cyclophyllidea (Lühe 1910) a new family, Nematotaeniidae, has been created for the reception of *Nematotaenia dispar*. This classification does not take into consideration certain essential characters of development and morphology, which relate it more closely to other forms.

Fuhrmann (1908:29) and Ransom (1909:88) have placed Nematotaenia in the subfamily Paruterininae, with six other genera: Paruterina Fuhrmann, 1916; Culcitella Fuhrmann, 1916; Rhabdometra Kholodkovski, 1900; and Biuterina Fuhrmann, 1902. Following is Ransom's diagnosis of the subfamily (Ransom, 1909:85): "Hymenolepididae; scolex usually armed, rarely without rostellum. A single set of reproductive organs in each segment. Uterus simple or double with a single parauterine organ or multiple with several parauterine organs, into which the eggs pass in the final stage of development of the segment. Adults in birds and amphibia." Paruterina Fuhrmann, 1906, is designated as the type genus.

Nematotaenia differs greatly from the other six genera included in this subfamily. This is evidenced by its cylindrical form, its two testes, as compared with the numerous and indefinite number in the other genera, the early degeneration of the uterus and its numerous parauterine capsules.

Jewell (1916), after describing Cylindrotaenia, creates a new subfamily for its reception, and includes Nematotaenia. This new subfamily is characterized as follows: "Cylindrotaenianae: Cylindrical Dilepinidae having one or two dorsally placed testes, ovary and vitellaria ventral, vitellaria dorsal to ovary. Proglottids distinct at the posterior end only. The uterus breaks down early and the embryos are later enclosed in parauterine capsules." The same writer considers *Taenia pulchella* Leidy as probably belonging to this subfamily.

It is evident from the previous description of the new form, that it belongs in this subfamily. However, because of the great differences in the number and arrangement of the parauterine capsules, it cannot be placed in either the genus Nematotaenia or the genus Cylindrotaenia. The definite, regular arrangement of the capsules in two parallel

rows would make it generically distinct. It is therefore necessary to create a new genus for the reception of this form, the diagnosis of which would read as follows: Distoichometra: Scolex unarmed, without rostellum. Body generally circular in cross-section, or nearly so. Genital pores alternating irregularly. Testes two in number, dorsal. Cirrus pouch approximately one and one-half times as long as broad. Ovary ventral. Uterus horseshoe-shaped, breaking up early into two parallel rows of egg-capsules, 4 to 6 in a row. Capsules hold 3 to 6 eggs each. After breaking up of uterus, capsules remain clustered together and do not become scattered throughout the parenchyma.

Type species *Distoichometra bufonis*, gen. nov., sp. nov., with characters of the genus; adult from the intestine of *Bufo lentiginosus*.

The writer wishes to express his thanks to Professor Henry B. Ward, under whose supervision the work was done, for his helpful criticisms and suggestions.

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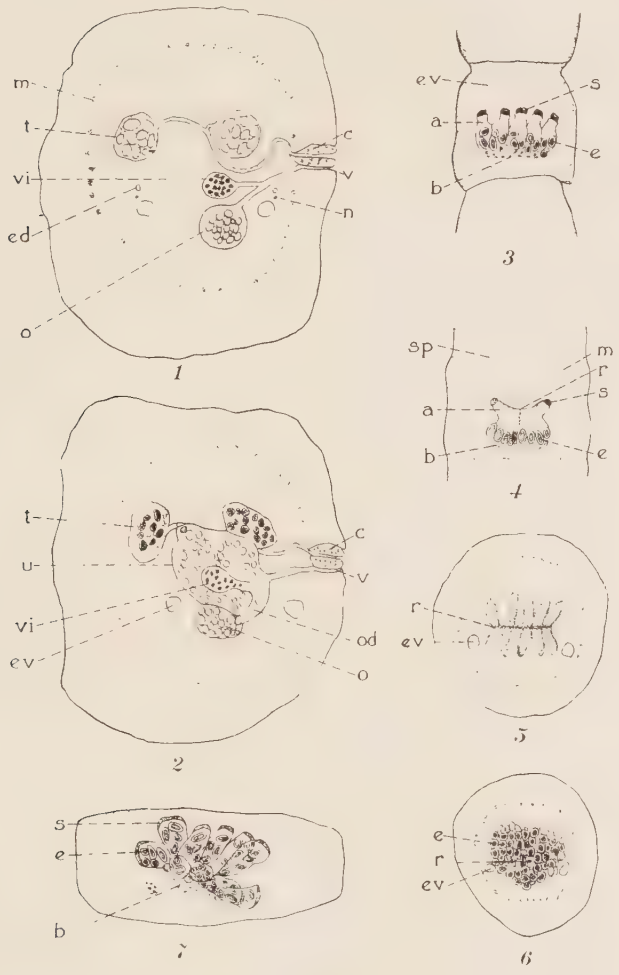


PLATE XIII

Explanation of Plate

*a*, Apical and *b*, Basal portion of parauterine organ; *c*, Cirrus pouch; *e*, Eggs in parauterine organ; *ed*, Dorsal excretory canal; *ev*, Ventral excretory canal; *m*, Longitudinal muscle; *n*, Longitudinal nerve; *o*, Ovary; *od*, Oviduct; *r*, Raphe; *s*, Gland-cell secretion; *sp*, Septum between proglottides; *t*, Testis; *u*, Uterus; *v*, vagina; *vi*, Vitelline gland.

Fig. 1.—Cross-section of mature proglottid, × 145.

Fig. 2.—Later stage than fig. 1, × 145.

Fig. 3.—Dorsal view of ripening proglottid with parauterine capsules. Toto mount.

Fig. 4.—Lateral view of slightly earlier stage than figure 3. Toto mount.

Fig. 5.—Cross-section through apical portion of parauterine organs. × 65.

Fig. 6.—Cross-section through basal portion of parauterine organs. × 65.

Fig. 7.—Ripe, detached proglottid. Toto mount.

# MICROSPORIDIA PARASITIC IN COPEPODS*

R. Kudo

While working on Cnidosporidia of various aquatic animals at Spring Valley, New York, during the summer of 1920, my attention was called to microsporidian infections in *Cyclops albidus* and *Cyclops fuscus*. Notwithstanding the fact that an enormous number of papers dealing with the occurrence, taxonomy and biology of several species of copepods have been published, it is strange to find but a few brief notes recording the occurrence of microsporidia-like parasites in the animals under consideration. Aside from one brief paper by Schröder (1914), all the others, which will be reviewed later, were published in Europe between 1887 and 1895 and the microsporidian nature of the forms described, is open to question. I have failed to find any record from any other land than Europe. In view of the circumstances, it seems worth while to describe the microsporidia which have come under my observation, and to review the old European records.

Although six species, i. e., *Cyclops albidus, C. fuscus, C. ater, C. phaleratus, C. prasinus* and *C. serrulatus,* were examined, only the first two species were found to be infected by the microsporidian parasites. In each collection, the number of individuals of *Cyclops albidus* predominated over the other five species. The absence of the infected animals in the latter four species may be due to the small number of individuals examined, and not to the specific difference in the host. It seemed to me that there could not be any special factor or factors that might have caused the infection to occur among the first two host species.

Before the examination for the microsporidian parasites, each host animal was placed on a slide, and identified by means of Marsh's key (1918). The animal was then slightly pressed under a cover glass, and examined microscopically in either fresh and stained smears or section preparations. The methods of fixation and staining, and also of the extrusion of the polar filament were exactly same to those which I have used in my previous studies (Kudo, 1920, 1920a).

*Nosema cyclopis* nov. spec.

Habitat: In the fat bodies and reproductive organs (?) of *Cyclops fuscus*. Spring Valley, New York (August).

Two, one male and the other female, out of twenty-two individuals collected from a creek on August 20, were found to harbor the Micro-

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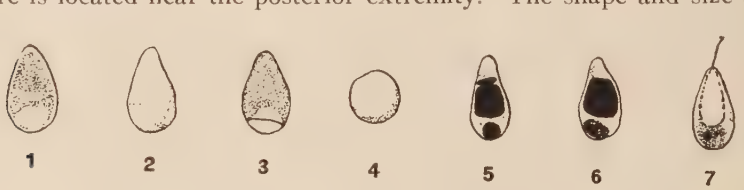
* Contributions from the Zoological Laboratory of the University of Illinois, No. 178.

sporidian. Six fixed specimens from Conger's Lake and twenty-four from the outlet of the latter were free from the infection.

The two infected animals were strikingly whitish opaque in color compared with the normal ones. The host did not suffer any decrease of the activity. This is probably due to the fact that the parasites do not attack the muscle cells. Concerning the effect of the parasitism upon the host body, I cannot make any definite statement as the number of the infected animals were small and they were not kept alive for any length of time under observation. However, there seems to be little doubt as to the fatal outcome of the infection.

The spores are pyriform (Figs. 1 to 7). The anterior end is rounded at its tip, while the posterior margin is broadly rounded. The spore membrane is very thin. The spore is less refractive than that of any species which I have studied up to the present. In cross-section, the spore is circular (Fig. 4). The broadest portion of the spore is located near the posterior extremity. The shape and size are




Figs. 1 to 7. Spores of *Nosema cyclopis* nov. spec. 1 to 3, fresh spores. 4, an optical cross-section. 5, a spore stained with Giemsa's stain. 6, a spore stained with Heidenhain's iron hematoxylin. 7, a spore mechanically compressed and stained with Giemsa's stain, the greater part of the polar filament is not shown. × about 2350.

constant. In the fresh state, there is always seen an oval clear space at the posterior end of the spore (Figs. 1 to 3). The rest of the cavity of the spore is filled with finely granulated cytoplasm. When stained (Figs. 5 and 6), there appear two deeply stained masses in the spore, a sporoplasm which looked as a clear space in the fresh spore and the coiled polar filament as was the case in *Thelohania magna* (Kudo, 1920) or *Nosema apis* (Kudo, 1920a). The polar capsule could be detected in some spores where the polar filament was extruded (Fig. 7). The polar filament seemed to be coiled up in a way similar to *Thelohania magna*. The doubly coiled condition which was recognized in *Nosema apis*, was not observed. Fresh spores measure 4.2 to 4.7$\mu$ long by 2.7 to 3$\mu$. The length of polar filaments extruded under the influence of mechanical pressure, fixed with sublimate alcohol and stained with Giemsa's stain, varies from 75 to 100$\mu$.

*Nosema infirmum* nov. spec.

Habitat: In the fat body, reproductive organs and muscle of *Cyclops albidus*. New City pond and the outlet of Conger's Lake (August and September).

This species was noticed in one out of twelve host specimens obtained from New City pond on August 29, and in twenty-one out of 153 collected in the outlet of Conger's Lake on September 6. The animals collected in the same creek from which *Cyclops fuscus* infected by *Nosema cyclopis* were obtained, proved to be free from the infection. The infected animals were as strikingly whitish opaque in color as in the case already mentioned. However, they showed a marked decrease in activity compared with normal ones. While the apparently uninfected individuals were hard to capture with a pipette, the infected animals were easily caught by the same means.

The effect of the Microsporidian upon the host body in this case seems to be fatal. In the collection from the latter locality, I found fifteen dead animals completely filled with the spores and extremely whitish opaque in appearance, which condition made such animals conspicuously visible against the brownish bottom soil of the aquarium


Figs. 8 to 17. Spores of *Nosema infirmum* nov. spec. 8 to 11, fresh spores. 12, an optical cross-section of a fresh spore. 13 and 14, young spores, stained with Giemsa's stain. 15, spore stained with Heidenhain's iron hematoxylin. 16, a spore stained with Giemsa's stain. 17, a spore mechanically compressed and stained with Giemsa's stain, the greater part of the polar filament is not shown. × about 2350.

in which they were kept. As no observations were carried out, due to the lack of time at that time, on the infected animals kept in the aquarium for any length of time, it is hard to determine the cause of the death of these *Cyclops albidus*. I am, however, led to consider that the Microsporidian infection was more or less directly responsible for the death of the host animals.

The spores are pyriform (Figs. 8 to 17). The anterior end is rounded at the tip. The posterior extremity is either more pointed or rounded than the anterior. The shape of the spore is however distinctly different from that of the species just described. The spore membrane is thin, but is slightly thicker than that of *Nosema cyclopis*. In cross-section, the spore is circular (Fig. 12). The broadest part

passes through the middle of the long axis of the spore. The size and shape vary to a certain extent. In the fresh state, there is seen a clear space which is either oval or irregularly triangular in form, at or near the posterior extremity of the spore (Figs. 8 to 11). The rest of the contents of the spore appear finely granulated. When stained, the cytoplasm is seen to be located at the posterior end where a clear space was noticed in the fresh state. The polar filament is more distinctly visible even in unextruded condition than the former species (Figs. 15 and 16). The fresh spores measure 5.6 to 6.4$\mu$ long by 3$\mu$ broad. The length of the polar filament, as determined in the same way mentioned in the first species, varies from 90 to 115$\mu$.

Observations upon the schizogony and sporogony are insufficient and I cannot give satisfactory full accounts of the changes that take place. As far as the observation up to the present date, is concerned, the vegetative forms of the two species, the schizonts as well as sporonts, can not be distinguished from each other unless spores are found with them, in which case, the distinction between the two species is comparatively easily done.

The youngest schizonts are rounded bodies, each with a single deeply stained chromatic mass. The body increases in size as the nucleus multiplies repeatedly, forming spherical, oblong or elongated bodies with 2, 3, 4, 5 or 6 nuclei. The ultimate products seem to be uninucleate rounded sporonts. Each sporont develops into a single spore which characterizes the genus Nosema. The development seems to be much different from *Thelohania magna* or *Thelohania illinoisensis*, but similar to that of *Nosema bombycis* (Kudo, 1916) or *Nosema baetis* (see my account).

The difference in the form, appearance and size of the spores of the two species, leads me to record them as two distinctly different species.

An examination of previous records shows that in every case the absolute proof of the microsporidian nature of the parasites, which is, above all, the presence of a polar filament in each spore, is lacking. Strictly speaking, therefore, one can not say whether any Microsporidia were previously found in copepods or not, without reexamining the preparations if such can be obtained.

The nature of "Pilzsporen" found and mentioned by Claus (1863: 87) in the body cavity of *Cyclops* species, remains undetermined, although he seemed to have seen bodies similar to the spores of *Nosema bombycis*.

Moniez (1887) described briefly the following three species, two of which were later studied by Pfeiffer (1895) and the other by Schröder (1914).

*Nosema* (?) *parva* Moniez

1887, *Nosema parva,* Moniez, 1887: 1313.

1895, *Glugea leydigii,* Pfeiffer, 1895: 83, 86.

Habitat: *Cyclops* spp. at Lille and Weimar.

Pfeiffer states that the species invades the fat bodies and reproductive organs.

Vegetative form: Moniez simply states that the sporogenic masses are relatively voluminous. Pfeiffer describes "cysts" with spores are rounded or elongated. The "cyst" with macrospores is about half the size of that with microspores.

Spore: After Moniez: oval, with a clear space regularly at one end. Size 3.5$\mu$ by 2$\mu$. After Pfeiffer: pyriform, with a clear spot at rounded end. Size 8$\mu$ by 5$\mu$.

Remarks: The identity of the two forms is very doubtful because of the unusual difference in size of spores. The description is so brief and incomplete that one cannot place the species definitely to any genus. The original nomenclature is retained.

*Thelohania acuta* (Moniez)

1887, *Microsporidium acuta,* Moniez, 1887: 1314.

1914, *Thelohania acuta,* Schröder, 1914: 324-327.

Habitat: *Cyclops gigas* and *Daphnia pulex* at Lille and in Germany.

Schröder states that the infection was chiefly noticed in the fat bodies.

Vegetative form: Not described.

Spore: After Moniez: The spore terminates in a sharp point, and measures 5$\mu$ long by 2$\mu$ broad. After Schröder (on fixed specimens): Elongated pyriform; one end terminates in a blunt point, while the other is rounded. Circular in cross-section. A pyriform polar capsule not longer than the half of the spore, is seen at the anterior half. The rest of the spore is filled with cytoplasm which contains a spherical vacuole at the posterior end. The polar filament could not be extruded. Size same to that measured by Moniez.

*Thelohania virgula* (Moniez)

1887, *Nosema virgula,* Moniez, 1887: 1313.

1895, *Glugea virgula,* Pfeiffer, 1895: 86.

Habitat: *Cyclops* spp. at Lille and Weimar.

Vegetative form: Moniez states simply that the sporogenic masses reach 30$\mu$ by 20$\mu$.

Spore: After Moniez: pyriform, one end rounded, the other sharply pointed. Anterior part is often bent to one side. A large vacuole at the rounded end. Size 8$\mu$ by 3$\mu$. After Pfeiffer: spores (eight in his figure) are always arranged in a stellate form. Size 8$\mu$ by 5$\mu$.

Remarks: The identity of these two forms is again doubtful. Pfeiffer's figure suggests that the species, if it is a Microsporidian, may belong to the genus Thelohania. It is provisionally placed there.

A parasite seems to have been observed by Schmeil (1891), Schewiakoff (1893) and Pfeiffer (1895).

*Glugea schmeili* Pfeiffer

1891, Myxosporidia, Schmeil, 1891: 19-21.

1893, Myxosporidia, Schewiakoff, 1893: 15-25.

1895, *Glugea schmeilii,* Pfeiffer, 1895: 84-86.

Habitat: *Cyclops* sp., *Diaptomus coeruleûs* and *D. salinus* at Halle and Weimar.

Schewiakoff states that the species forms conspicuous masses in the body cavity and the spores are found "on" the muscle. Both Schmeil and Schewiakoff remarked on the opacity of the body of the infected animals.

Vegetative form: Schewiakoff studied the continuous changes in living animals using apochromatic objective 4 mm., and states as follows: The amoeboid forms are found in the body cavity of the host. They are uninucleate. The cytoplasm is finely granulated, and forms hyaline lobose pseudopodia from any part of the surface. A nucleus and a contractile vacuole are present. Size varies from 7 to 20$\mu$ long by 3 to 6$\mu$ broad. They creep around on the epithelial and muscle cells. They become encysted. Plasmotomy of two or three individuals was frequently noticed. In the cyst, the nucleus or nuclei divides into a large number. Each daughter nucleus becomes the center of a spore.

Spore: After Schewiakoff: oval, measuring 3.2 to 4$\mu$ in length. At the broader end, a homogeneous, spherical (1.6$\mu$ in diameter) and refractive nucleus is located. Each "spore" further divides into two, the nucleus undergoing mitosis (number of chromosomes eight).

Remarks: It is highly doubtful if the amoebula with a contractile vacuole, mentioned by Schewiakoff, is a stage in the life cycle of the present paratite. Assuming that this is a Microsporidian, then it should be placed in the genus Glugea, as Pfeiffer did.

The following two more species described by Fritsch (1895) who thought them to be Microsporidia, and placed them in the genus Glugea, are much more doubtful forms than the preceding species.

*Glugea colorata* Fritsch

1895, *Glugea colorata,* Fritsch, 1895: 80-81.

Habitat: *Diaptomus gracilis* in Austria. The parasite formed masses of olive green or burnt sienna in color.

"Cyst" contained only 5 or 6 spores.

*Glugea rosea* Fritsch

1895, *Glugea rosea,* Fritsch, 1895: 81.

Habitat: *Cyclops strenuus* in Austria (June). One host animal rose-colored was observed.

Spore: Two kinds: one, smaller, oval with a somewhat pointed end and yellowish in color; the other, larger and elongated pyriform with or without vacuole.

Compared with these six ambiguous species of Microsporidia (?), the two American forms described differ greatly. Hence I have designated them by new specific names. On obtaining more material, I shall try to work out the life history of the Microsporidia.

## SUMMARY

1. Two new Microsporidia, *Nosema cyclopis* nov. spec., parasitic in *Cyclops fuscus,* and *Nosema infirmum* nov., parasitic in *Cyclops albidus,* are described.

2. The effect of the infection of *Nosema infirmum* upon its host body, seems to be fatal.

3. The former papers which recorded the occurrence of Microsporodia-like parasites in copepods, are reviewed.

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# EFFECTS OF SECRETIONS OF CERTAIN PARASITIC NEMATODES ON COAGULATION OF BLOOD.[1]

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## INTRODUCTION

The fact that certain parasitic nematodes, especially some members of the family Strongylidae, a group that includes the hookworms, have the power of lacerating the intestinal mucosa, places these parasites in the category of serious pathogenic agents. In considering the question of their pathogenicity, in addition to the damage done by the abstraction of blood and by the mechanical injury to the mucosa of the intestine, caused by their bites, including the entrance of bacteria, it is important to consider the possible effects of the secretions of the worms on the intact as well as on the injured intestinal mucosa and the general effects on the host of the absorption of these secretions into the circulation. Among the toxic products elaborated by these nematodes substances that retard coagulation of blood have been found in certain species. That these substances are responsible for the persistent oozing of blood from wounds inflicted by hookworms and related nematodes, appears probable.

A record and discussion of the writer's experiments on effects of extracts of certain nematodes on coagulation of blood is given in the following pages. No attempt has been made to correlate the results of these experiments with theories of blood coagulation.

## REVIEW OF LITERATURE

That the secretions of certain nematodes have the power of retarding coagulation of blood *in vitro* was first shown by Loeb and Smith in 1904. These investigators found that extracts of *Ancylostoma caninum* in physiological salt solution inhibit the coagulation of dogs' blood *in vitro* for periods which vary with different samples of blood, the maximum period of delay in coagulation observed by these writers being about twenty-four hours. Loeb and Smith found, moreover, that the substance involved in this process is present in the anterior half of the worms and is completely absent in the posterior half. The substance was found by these writers to be highly resistant to heat since

1. This paper was read before the Helminthological Society of Washington, on November 20, 1920, at the School of Hygiene and Public Health of Johns Hopkins University, Baltimore, Md.

2. Resigned, December 15, 1920.

fifteen minutes' boiling merely weakened but did not destroy its power to inhibit coagulation of blood.

Although the work and conclusions of Loeb and Smith are questioned by Liefmann (1905), experimental work by Loeb (1906) and Loeb and Fleisher (1910) showed quite conclusively the presence in the anterior portion of the body of *Ancylostoma caninum* of a substance that retards coagulation of dogs' blood, and confirmed the conclusions of the earlier work of Loeb and Smith.

Weinberg (1907) in the course of his investigations on effects of extracts of horse strongyles, belonging to the genus Strongylus, on the blood of the horse, found that physiological salt solution extracts of triturated specimens of these worms inhibited coagulation of horses' blood, since mixtures of the freshly drawn blood and extracts in question were still uncoagulated after four days.

Aside from the experimental work with extracts of worms belonging to the genera Ancylostoma and Strongylus, there appears to exist some evidence in favor of the view that the fluid which occurs in the body cavity of worms belonging to the genus Ascaris has the power of inhibiting to a certain extent coagulation of blood *in vitro*. Leroy (1910), experimenting with dogs, and Weil and Boyé (1910), experimenting with rabbits, found that the blood of animals which had been injected with the body fluid of ascarids coagulated more slowly than the blood of non-injected animals, but Weil and Boyé failed to observe that the fluid had any effect on the coagulation of rabbits' blood *in vitro*. Flury (1912), on the other hand, found that the fluid delayed coagulation of dogs' blood and human blood *in vitro*.

## EXPERIMENTS BY THE WRITER

Experiments by the writer have been made with physiological salt solution extracts of *Strongylus vulgaris, Strongylus edentatus, Bustomum phlebotomum, Bustomum trigonocephalum, Stephanurus dentatus, Oesophagostomum columbianum, Dictyocaulus filaria, Haemonchus contortus, Ascaris lumbricoides, Ascaris equorum,* and *Belascaris* sp. The extracts in question were prepared from specimens collected shortly after the death of the host. The specimens were washed in physiological salt solution, dried between layers of filter paper and exposed to room temperature or to a temperature of 37° C. until they became sufficiently crisp to be pulverized. A quantity of powder was then added to physiological salt solution in a test tube, the contents were thoroughly shaken and allowed to remain in a refrigerator at a temperature of about 10° C. for about twenty-four hours. Before being used in experiments, extracts prepared as outlined above were filtered through ordinary filter paper. In nearly all experiments referred to in the following pages about 0.1 gm. of powder of the

dried parasite in question was added to each cubic centimeter of physiological salt solution. Equal parts of freshly drawn blood and of extract were used in each experiment. Each experiment was controlled by adding to a quantity of the blood that was used in the test an equal volume of physiological salt solution.

Weinberg's conclusions concerning the presence in worms belonging to the genus Strongylus of a substance that inhibits coagulation of blood were confirmed. Extracts of *Strongylus edentatus* and of *Strongylus vulgaris* inhibited coagulation of rabbits' blood for periods ranging from thirty minutes to sixty minutes, as compared with controls. Rabbit blood in contact with extracts of specimens of *Strongylus edentatus* that had been preserved in alcohol for several weeks showed no delay in coagulation. The substance in these worms that delays coagulation of blood is evidently less potent for rabbit blood than for horse blood, which probably indicates that it has a selective action on the blood of its host. That this substance is not limited to the anterior part of the worm, as is the case in worms of the genus Ancylostoma, was shown by the following experiment:

The anterior portions (roughly about one-third of the total length of the worms) of seven dried specimens of *Strongylus edentatus* were triturated in a mortar and extracted in one cubic centimeter of physiological salt solution. The remaining posterior portions of these specimens were also triturated and extracted in an equal quantity of salt solution. Freshly drawn rabbit blood in contact with the above extracts remained uncoagulated one hour, whereas the control was coagulated in five minutes. The blood in the tube containing the extract of the posterior portion was still fluid when that in the tube containing the extract of the anterior portion was beginning to coagulate, but the difference between the rapidity of coagulation of the two samples of blood was only five minutes.

A series of experiments was performed with extracts of cattle hookworms *(Bustomum phlebotomum)*. Experiments 1 to 5 were performed with five different samples of freshly drawn cattle blood.

Experiment 1: The blood remained uncoagulated for thirty minutes. The blood in the control tube coagulated in ten minutes.

Experiment 2: The blood remained uncoagulated two and one-half hours. The blood in the control tube became coagulated in ten minutes.

Experiment 3: The blood remained uncoagulated for two and one-half hours. The blood in the control tube became coagulated in fifteen minutes.

Experiment 4: The blood remained uncoagulated three and one-half hours. The blood in the control tube became coagulated in fifteen minutes.

Experiment 5: The blood was still uncoagulated after twenty-four hours. The blood in the control tube became coagulated in ten minutes.

Experiment 6: Rabbit blood was used in this experiment. The blood remained uncoagulated for fifty minutes. The blood in the control tube was coagulated in seven minutes.

In the series of experiments upon cattle blood it was observed that only a portion of the blood actually coagulated. In the control tubes the blood clot was from two to three times as large as that in the tubes containing the extract. The latter showed a heavy sediment of erythrocytes, whereas the control tubes showed but a slight sediment of red blood corpuscles.

Experiments with extracts of a closely related species, namely, *Bustomum trigonocephalum,* a hookworm parasitic in sheep, yielded the following results: Two samples of rabbits' blood showed a delay in coagulation of twenty minutes as compared with the controls, and one sample of cattle blood showed a delay of forty-five minutes as compared with the control.

In order to determine whether the substance in the worms that inhibits coagulation of blood is readily soluble in salt solution, powder used in experiments 1 to 6 which had been extracted once was re-extracted and tested on samples of cattle blood with the following results: In two cases no effects were produced, since coagulation occurred in the controls and in the tests at the same time. In one case coagulation was delayed ten minutes as compared with the control and in another case it was delayed fifteen minutes, thus showing that the first extraction removed practically the entire anticoagulin from the parasite material.

Extracts of the stomach worm *(Haemonchus contortus)* were tested on ten samples of sheep blood, on five samples of cattle blood, and on several samples of rabbit blood. In nearly all cases the blood in contact with the extracts coagulated more slowly than the controls, but the maximum delay in coagulation of blood in contact with *Haemonchus contortus* extract as compared with the controls was about fifteen minutes.

Extracts of the kidney worm of swine *(Stephanurus dentatus),* of the lungworm of sheep *(Dictyocaulus filaria),* and of the gapeworm of poultry *(Syngamus trachealis)* were tested on three samples of sheep blood with negative results.

Extracts of *Oesophagostomum columbianum,* the nodular worm of sheep, prepared by macerating twelve fresh specimens in 2 c.c. of physiological salt solution, produced no effect on two samples of rabbit blood and one sample of cattle blood.

The negative results obtained in these experiments, as well as the weakly positive results obtained with *Haemonchus contortus,* serve as a control on the specificity of the reaction with extracts of species of Ancylostoma, Bustomum, and Strongylus, and show quite conclusively that the substance or substances in the worms which inhibit coagulation of blood are specific anticoagulins physiologically related to hirudin and certain snake venoms, and not merely mixtures of

proteins in solution. While solutions of proteoses, of trypsin, of pepsin, and extracts of tissues are known to retard coagulation of blood when injected into the living animal, they have been shown not to delay coagulation of blood *in vitro,* and hence the effects on coagulation of blood *in vitro* produced by extracts of certain nematodes cannot be ascribed to such substances.

A series of experiments with *Ascaris lumbricoides* fluid and rabbit blood yielded the following results: Three to five drops of fluid of *Ascaris lumbricoides* from swine in contact with ten drops of blood produced a delay in coagulation of fifteen minutes as compared with the control. A mixture of eight drops of fluid and ten drops of blood remained uncoagulated thirty-five minutes longer than the control. A mixture of ten drops of fluid and ten drops of blood showed a delayed coagulation of forty-two minutes as compared with the control.

Extracts of *Ascaris equorum* and of *Belascaris* sp. were tested on three samples of sheep blood with weakly positive results, i. e., blood in contact with these extracts remained uncoagulated five to fifteen minutes longer than the controls.

These experiments confirm Flury's results with human blood and dog blood and show that the fluid that is present in the body cavity of *Ascaris lumbricoides* delays to a certain extent coagulation of blood *in vitro.*

## DISCUSSION

That certain nematodes secrete substances that have toxic properties and that are absorbed by the host is a view which has been advanced by a number of investigators. In addition to the toxic principle discussed in this paper, other specific toxic substances, especially hemolysins, have been shown to occur in several species. It is probable that the toxic secretions of nematodes, like snake venoms, are a complex of a number of different principles, such as hemolysins, anticoagulins, and one or more systemic poisons.

It is of interest to note that nematodes that contain anticoagulins also contain hemolysins.[3] The former are probably distinct from the latter chemically as well as physiologically. The hemolysin found in species of Ancylostoma is destroyed by heating at 55° C. for several minutes (Whipple, 1909; Schwartz, 1920), whereas the anti-coagulin in these worms resists boiling (Loeb and Smith, 1904). Further studies on the properties of anticoagulins from nematodes would probably yield interesting comparisons with those of hemolysins. Investigations concerning the possible presence of hemolysins in nematodes that lack anticoagulins would also be of interest.

3. A brief account of hemolysins from parasitic worms is given in a recent paper by the writer (Schwartz, 1920).

It is conceivable and by no means improbable that substances in nematodes which delay coagulation of blood may be of etiological significance in the pathology of nematode diseases. Loeb and his collaborators are inclined to the view that the oozing of blood from the wounds inflicted by hookworms, rather than the absorption by the host of a hemolysin elaborated by the parasites, accounts for the anemia that occurs in cases of infestation with these parasites. That the anticoagulin which has been shown to occur in the anterior portion of these parasites is responsible for the persistent hemorrhages in question appears to be a warranted conclusion. In this connection it is of interest to observe that markedly strong anticoagulins, so far as is known, occur only in nematodes belonging to the family Strongylidae, the members of which commonly produce pronounced anemia in the host.

## SUMMARY

1. The substance in species of Strongylus that inhibits coagulation of blood is present in the posterior as well as in the anterior portion of the worms.

2. *Bustomum phlebotomum,* a hookworm parasitic in cattle, contains a substance soluble in salt solution that inhibits coagulation of blood for considerable periods which vary with different samples of blood. A closely related species, *Bustomum trigonocephalum,* contains a similar anticoagulin.

3. Salt solution extracts of *Haemonchus contortus* cause but a slight delay in coagulation of blood. Extracts of *Syngamus trachealis, Dictyocaulus filaria,* and *Stephanurus dentatus* do not retard coagulation of sheep blood. Extracts of *Oesophagostomum columbianum* do not retard coagulation of rabbits' blood.

4. The body fluid of *Ascaris lumbricoides* inhibits coagulation of blood to a moderate extent. Extracts of *Ascaris equorum* and of *Belascaris* sp. have a slight effect on coagulation of sheep's blood.

5. In view of the fact that the delay in coagulation of blood due to extracts of nematodes occurs *in vitro,* that it varies with extracts of different species of worms, and that extracts of certain species produce no delay in coagulation, it may be concluded that specific substances, other than proteins in solution, must be involved.

6. The substances in question appear to be physiologically related to hirudin and snake venom, and like the latter are probably part of a complex of toxic principles.

7. So far as present knowledge goes, nematodes which contain substances that inhibit coagulation of blood to a marked degree are zoologically related, belonging to the family Strongylidae, the mem-

bers of which have a buccal capsule adapted to lacerating the intestinal mucosa.

8. That the injection of their secretions into the intestinal mucosa, by certain biting nematodes, resulting in minute hemorrhages, is of etiological importance in nematode diseases appears very probable.

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# A MICROSPORIDIAN OCCURRING IN THE SMELT

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In the course of taxonomical studies on the smelts, Dr. A. C. Kendall and D. R. Crawford of the Bureau of Fisheries have frequently encountered a very characteristic infection in these fishes. This infection I find to be caused by one of the Microsporidia, belonging to the genus Glugea.

Infections at various stages of development were available. Apparently, the intestine is the primary seat of the parasite. Affected fishes are characterized by the appearance of more or less numerous cysts in the viscera, and generally all the cysts in a fish are of approximately the same size. The latter may vary from microscopical dimensions to 3 mm. in diameter. Early stages show them in the wall of the intestine where their white color makes them conspicuous even when still small. At this time they are located in the mucosa, below the epithelium of the villi (Fig. 2). As growth proceeds, they push through the muscular coat of the intestine, and then come to lie immediately under the peritoneum. An extreme but common manifestation of such cyst development is shown in Figure 1. The entire length of the intestine from below the stomach to within a short distance of the anus is here taken up with cysts, and it becomes a puzzle how it can function under such conditions. Cysts also occur in the liver and the gonads, but not one was found in the stomach proper, kidney or heart.

The distribution of affected smelts is a very interesting one. Such fishes were found in Lake Massabesic, N. H.; Sunapee Lake, N. H., near Dennysville on the extreme northern portion of the Maine coast, and Casco Bay on the southern Maine coast. The smelts in Sunapee Lake were introduced some years ago from Squam Lake, N. H., from which no records are available. It is to be noted that the specimens from the Maine coast are typical smelts which live in salt water and ascend fresh water streams at a certain time each year. Those from the lakes mentioned, however, are purely fresh water forms and never come in contact with salt water. Aside from other considerations, dams and other obstructions would make journeys from the sea into these lakes a physical impossibility. It may also be remarked that Dr. Kendall (paper in preparation) believes that these fresh water smelts are taxonomically distinct from the salt water form, *Osmerus mordax*.

Nevertheless, the Microsporidian parasites seem to be identical in both fresh and salt water smelts. Whether the parasite became estab-

lished after the several types of smelts had evolved, or whether it was present originally and underwent no morphologic changes in distinction to its host, must be left unanswered at present.

The rate of infection may be very high, as is manifested by the following data:

Lake Massabesic, N. H.: 38 out of 71 infected—over 53%. (Mild infections were not counted in this instance.)

Sunapee Lake, N. H.: 29 out of 103 infected—over 28%.

Dennysville, Me.: 1 out of 64 infected—over 1.5%.

Casco Bay, Me.: 48 out of 306 infected—over 16%.

In Casco Bay the distribution is very general, and affected fishes were caught at Mosiers Island and Freeport (Harraseeket River, Mast Landing Creek, and Porters Landing Creek). Collections were made in 1904, 1907, and 1915, and generally either in spring or autumn.

Adult fishes are only rarely parasitized, the highest rate of infection being found among immature fishes of approximately 10 cm. in length (generally speaking, about a year before maturity). Fishes of the latter size are very much more numerous than adults. Possibly the scarcity of parasitized adults is due to the fact that the majority of infected immature fishes die, leaving only those that escape infection to attain maturity. It must not be forgotten, of course, that such a disparity in the numbers of young and mature fishes is encountered to some degree in all other species of fishes, where parasitism may not be instrumental at all.

Glugea sp.

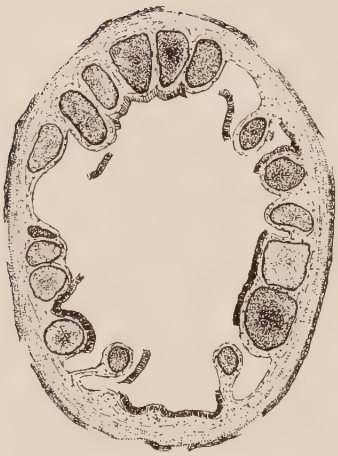
Cysts vary in size from microscopic dimensions to 2 or 3 mm. in diameter. As in other species of Glugea, sporonts, sporoblasts, and ripe spores may all be found in a single cyst, with the earliest stages near the periphery. Spore formation seems to follow the same lines as described for *Glugea anomala* by Stempell (1904), and Awerinzew and Fermor (1911).

Dimensions of spores: length = 4 to 4.5$\mu$; width = 2 to 2.5$\mu$.

The preservation of the material rendered a study of the nuclear conditions in the spores impossible, although other developmental stages were not badly fixed. The giant vegetative nuclei which have been the basis of much dispute, are very numerous at the periphery of developing cysts. To all appearances, they give rise to sporonts, as Stempell and Awerinzew and Fermor have maintained, and are not hypertrophied tissue nuclei of the host (Schuberg, 1910, and Schroeder, 1909).

Unlike *Glugea anomala*, the parasite is specific for the smelts and does not affect even other Salmonidae inhabiting the same waters. (It is indeed open to question whether the Microsporidia of Gasterosteus could be transmitted to Gobius, although Stempell believes that in both species of fishes, which are of widely different families, *Glugea anomala* is concerned.) Again, the size given above is fairly constant, and no such extreme variation as described in *G. anomala* could be

1



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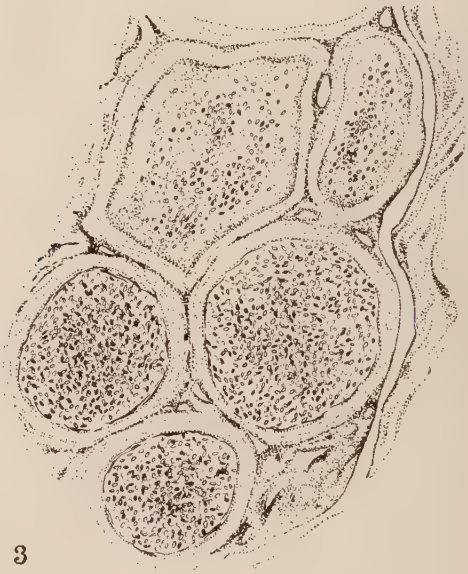

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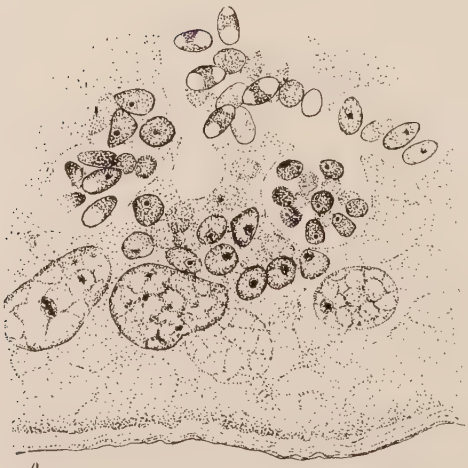

4

5

PLATE XIV

observed. Muscles and connective tissue were found not to be subject to invasion, which also is a point of difference from that described species.

Microspore infections of the smelt have also been reported for North America by Mavor (1915) and Linton (1901). The latter records sporocysts in the intestine of this fish, but does not go into detail any more than Mavor, who contents himself with the statement that the microspore concerned in his case was apparently *Glugea stephani*. The measurements of the latter, which is typically a parasite of the flatfishes, differ definitely from the parasite which I have described—3 by 1.5 mm. (Johnstone, 1901). Its mode of occurrence in the intestine and its life history are, however, markedly similar, and it seems probable that both Mavor and Linton were dealing with the same parasite as that discussed in this paper.

A form which in both size and occurrence corresponds almost exactly to the one I have described, was found by Weissenberg in the European smelt, *Osmerus eperlanus,* and named *Glugea hertwigi.* The dimensions given for this form are 4.6 to 5.4 by 2.3 $\mu$, which measurements are very close to those of the American form. Weissenberg's measurements were taken from fresh specimen, which may account for their slightly larger proportions. The occurrence of the same parasite in European and American species of the smelt would furnish an interesting parallel to the case of *Myxidium lieberkuehni* which according to Mavor occurs in both the European and the American pike.

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## Explanation of Plate

1. Smelt showing advanced infection of liver and intestine. Reduced one fifth.

2. Cross section of intestine showing an early infection. 20 ×.

3. Portion of parasitized testis. Objective: 32 mm. Eyepiece: 6 ×.

4. Periphery of developing cyst showing giant nuclei and developmental stages. Objective: 1.5 mm. Eyepiece: 10 ×.

5. Spores. Objective: 1.5 mm. Eyepiece: 15 ×.

# THE FIRST INSTAR OF *WOHLFAHRTIA VIGIL* WALKER

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In the September number of this journal Dr. E. M. Walker (1920) published an account of the larval structure and habits of *Wohlfahrtia vigil*. As the first instar has not yet been described the following may be of interest:

On July 9, 1907, in Ithaca, N. Y., the writer captured a female specimen, which when placed in the cyanide bottle, immediately deposited several larvae on the side of the glass. The species is therefore larviparous, like other Sarcophagids. As the adult of this species has several striking characteristics, it was possible, even at that date, and before the appearance of Aldrich's monograph (1916) to determine it by means of the original description of Francis Walker (1849), under the name of *Sarcophaga vigil*. Though not common in Ithaca, I have taken a few specimens of the fly nearly every season since 1907, and always in a similar situation, that is, on a cement walk, in the bright sunshine, about midday, during June, July and August.

The larva of the first instar measures 2.3 mm. in length, by 0.4 mm. in width in the region of the fifth or sixth abdominal segment. The upper pair of tubercles (or antennae of Portchinsky) of the pseudocephalon, are well defined, 30$\mu$ in length, two-segmented, the first segment about as long as broad, the second somewhat longer than broad, and conical. The mandibular hooks are slender, sharply pointed, and much curved, though less so than those figured for *Wohlfahrtia magnifica* Schiner by Portchinsky (1884). He states that in the first instar of the last mentioned species there is a large median hook placed a little higher than the laterals. In *W. vigil* there is no indication of this median hook. As it is scarcely conceivable that so experienced an observer as Portchinsky should mistake a portion of the pharyngeal skeleton for a median hook, we must conclude that *W. magnifica* differs from the American species in this particular. As in the first instar of the house fly larva the anterior spiracular processes are lacking. The posterior spiracles are in a pit and each has two slits which lie parallel to each other, approximately perpendicular to the horizontal plane, supposing the larva to be lying ventral side down in a horizontal position. The spinules on the body are rather larger and more numerous than indicated by Walker (1920) for the second and third instars, though less widely distributed than described by Portchinsky (1875) for *W. magnifica*. In my specimens the anterior third of the second

segment, and the anterior fourth of the third, fourth and fifth segments are each provided with a uniform spinule band. On the succeeding segments the bands occupy about one-fourth the width of a segment, are placed over the incisures and all are more or less interrupted by clear, transverse areas, corresponding to the folding at the incisures. The incisure cuts the fifth segment near its anterior margin, but the bands at the posterior end of the body are cut by the incisures nearer their middle. The clear areas are more numerous in the posterior bands, but their arrangement does not appear to be significant since this differs in distribution in different specimens. The spinules in the bands at the cephalic end are somewhat larger than those in the posterior bands.

The first instar, on the whole, is therefore more distinctly spinose than the second or third, as illustrated by Dr. Walker, for this species, but is less spinose than the European species described and figured by Portchinsky.

REFERENCES

Aldrich, J. M. 1916.—Sarcophaga and its Allies in North America. The Thomas Say Foundation.

Portchinsky, J. 1875.—Krankheiten, welche im Mohilew'schen Gouvernement von den Larven des *S. Wohlfahrti* entstehen, und deren Biologie. Horae Soc. Ent. Ross., 11: 122.

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Walker, E. M. 1920.—*Wohlfahrtia vigil* (Walker) as a human parasite. Jour. Parasitol., 7: 1.

Walker, Francis. 1849.—List of the Dipterous Insects. See *Sarcophaga vigil*, part 4, page 831.

# NOTES

## A NOTE ON LONGEVITY OF LARVAL TICKS

Some time about the last week of July, 1919, a small boy living at our Biological Station brought me a number of engorged ticks which he had taken from the ears of his dog. I placed two of the largest in a small wooden box with a tight cover. In two or three days they began laying eggs. This process continued for several days when the ticks and about half the eggs were taken out of the box. Some time near the first week in August, the eggs began hatching and all were hatched by about the middle of August. There were then several hundred newly hatched ticks in the box ready and waiting to be fed, but I carefully refused to be the victim.

The box was then left closed until November 14, 1919, when it was opened and a large number were still alive and active. In fact their numbers and activity prevented the making of any estimate as to how many were actually living. On December 28, 1919, the living larvae were still numerous as was also the case on January 4, 1920. By February 25, 1920, the numbers were so reduced and the activity so diminished that it was possible to estimate fairly satisfactorily that about fifty still lived. On March 11, 1920, only a few were alive and they were rather inactive. On April 9, 1920, none could be found showing signs of life.

The dozen or so which survived this period of seven months without ever having fed show very clearly that the perpetuation of this species is well provided for by qualities of endurance as well as by great numbers of young. One is led to wonder what changes occurred in form and function of organs of these animals during the ordeal. In some respects the tick would appear to be an organism unusually favorable for certain studies in nutrition.

Read at the Western Society of Naturalists, Seattle, June, 1920.

W. E. Allen.

Professor Ludwig von Graff died in Graz on December 8, 1920. While his work had been almost entirely in other fields he had written a small but valuable book on the parasites of domestic animals transferable to man and a large and extremely valuable work on The Turbellaria as Parasites and Hosts. The death of Professor Graff removes a commanding figure from the field of zoological research.

The library of the late Doctor A. J. Chalmers has been presented to the Royal Society of Medicine (England). It is to be known as the Chalmers Collection and to constitute the library of the new Section of Tropical Medicine and Parasitology. The British Medical Journal states that this is probably the finest collection of books on tropical medicine to be found anywhere.

Dr. Benjamin Schwartz has resigned as Assistant Zoologist in the Bureau of Animal Industry and has accepted the position of Professor of Protozoology and Parasitology in the University of the Philippines at Manila.

Dr. E. C. Faust has been given editorial charge of the department of Parasitology in the China Medical *Journal.*

## ERRATA

In The Journal (June, 1920). vol. VI, p. 175, line 4 from bottom, for 31 (the number of hooks) read 33.

In The Journal (December, 1920), vol. VII, p. 63, line 2, for *Grahamella protista* read *Grahamella talpae* n. g., n. sp. of Protista (Brumpt, 1911:514).